The Prevalence of *Blastocystis hominis* in Wadi Al-Shati Province, Libya

Awatif Mohamed Abdulsalam,* Abdul Hafeez Khan,** Abdul Gader Ajaili,*** Mohamed Al-Shebani,* and Gelani Salem Saad,*

Abstract:
The present work was planned to estimate the prevalence of *Blastocystis hominis* among random population of Wadi Al-Shati province. Stool specimens from 1000 randomized individuals were collected (during the period from the first of August 2007 to end of March 2008) from seventeen rural localities of Wadi Al-Shati province, presenting different sexes and ages (427 males and 573 females and aged from 1 to 90 years). Stool samples were examined using direct wet mounts and concentration method.
The overall prevalence of *B. hominis* was 21.20% (212/1000), the prevalence rates varied from 12.50 to 38.23% among random population of different localities of Wadi Al-Shati province. The highest positivity was found in Mansurah locality (38.23%) followed by Quttah locality (31.25%. Out of 15 samples examined in Ashkeda, none was found positive for *B. hominis*. Prevalence rates between males and females were 22.24% (95/427) and 20.41% (117/573) respectively. The difference was not statistically significant (P>0.05). The age group 11 to 20 years showed the highest rate of infection (24.00 %), and the lowest (13.77 %) was in 1 to 10 years.
Fecal specimens were examined by three direct wet mounts samples (normal saline, iodine and eosin stains) and formalin-ether concentration sedimentation smears in iodine stains. 183 samples (18.30%) were found to be positive for *B. hominis* in direct smears in normal saline, 197 samples (19.70%) were found to be positive in direct smears in iodine and 196 samples (19.60%) were found to be positive in direct smears in eosin stain for *B. hominis*. Concentration sedimentation detected *B. hominis* in 212 samples (21.20%). Comparative analysis of the results, showed no significant difference of sensitivity (P> 0.05) between direct smears and formalin-ether sedimentation for the diagnosis of *B. hominis* in fresh fecal material.

Introduction:
Blastocystis hominis occurs all over the world, but is commonly found in developing countries.[1,2] The occurrence of *B. hominis* infection has been reported is related to weather conditions with suggestion that infections are more common during hot weather or summer than winter or spring.[3,4]
The diagnosis of *B. hominis* in public health centers and laboratory is made by demonstration of typical vacuolar form in stool specimens. Practicing physician and gastroenterologists usually have low awareness of the association of *B. hominis* and human disease. These infections are overlooked in clinical laboratories. The Center for Disease Control and prevention also described it to be pathogenic protozoa.[5]
In Libyan Arab Jamahiriya, limited studies had been carried out for the diagnosis of *Blastocystis hominis*.[4,6-9] Moreover, detection of *B. hominis* in Libya is not routinely performed in laboratories, thus frequency of *B. hominis* and sources of infection are poorly known.
The results of recent studies in Sebha, Libya revealed that *B. hominis* was the most frequent isolate.[4,6] The prevalence of *B. hominis* among Libyan population in Wadi Al-Shati province of Libya has not been documented so far. *B. hominis* infection could be a public health problem among population in this region in future. The studies are required to detect infection of *B. hominis* by using routine laboratory method in the population of Wadi Al-Shati province, Fezzan, Libya for the health education program.

*) Medical Laboratory Department, Faculty of Engineering and Technology, Brack, Sebha University.
**) Medical Parasitology Department, Faculty of Medicine, Sebha University.
***) Zoology Department, Faculty of Science, Brack, Sebha University.

Materials and Methods:

Study area:

Wadi Al-Shati is one of the valley of Fezzan region situated in the zone of Wadi Al-Shati municipal branch, approximately at 27-28 N latitude in the south corner of Libya, (Fig. 1). The area of this branch measures about 57.160 Km² with population around 78532 reside in Wadi Al-Shati province. The stool samples were collected from seventeen rural localities (namely: **Ashkidah, Qirah, Brack, Zuwayah, Tamzawah, Ququm, Aqar, Mahrugah, Al-Desa, Al- Qardah, Quttah, Bergen, Zahra, Wanzarik, Tamssan, Mansurah** and **Idri**).

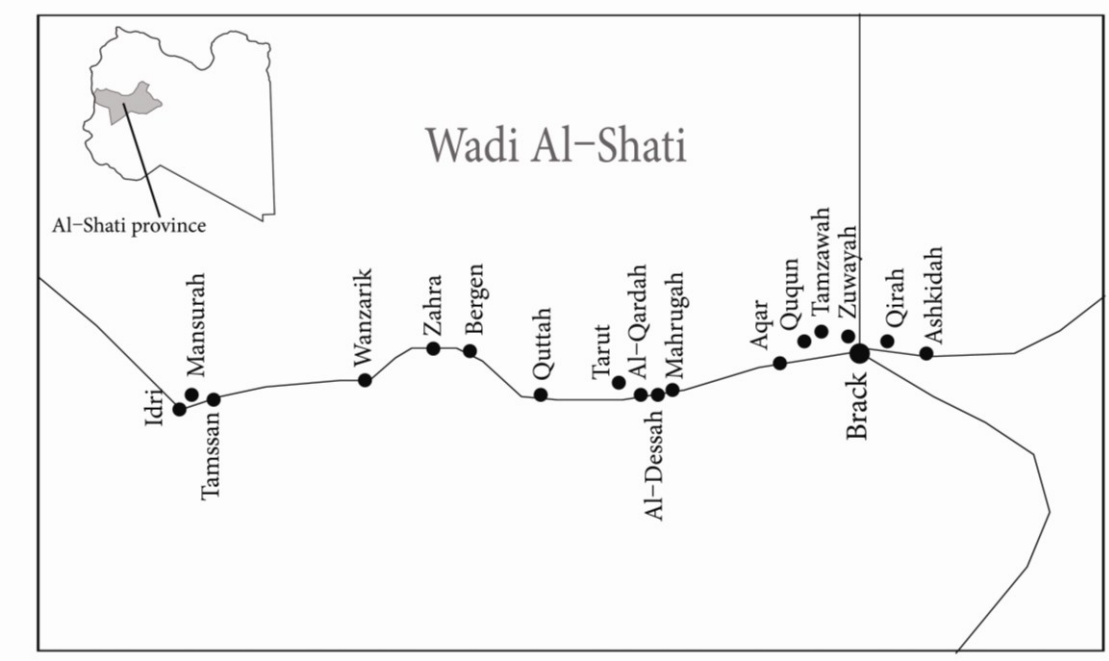

Fig.1: Geographic location of Wadi Al-Shati in Libya and different rural localities.

Sampling:

During the period from the first of August 2007 to the end of March 2008, a total of 1000 Stool specimens, presenting different sexes and ages (427 males and 573 females, aged from one year to 90 years) were collected randomly from individuals, who lived in seventeen rural localities of Wadi Al-Shati province for the detection of *Blastocystis hominis*. Stool samples were collected in closed and labeled disposable plastic containers. Data about age, sex, place of residence and Nationality were obtained from questionnaire given to the individuals.

Examination of stool specimens:

Each sample was processed and examined using direct fecal smears and the concentration technique for the detection of *Blastocystis hominis*.

1. Direct wet mount smears

Both direct wet unstained in normal saline and stained in Lugol's iodine and eosin preparations, were employed for the detection of *Blastocystis hominis*.

2. Direct saline wet preparation

The direct saline smear was prepared by mixing a small amount of feces with a drop of normal saline (0.85%) ; this mixture provided a uniform suspension under 22 X 22 mm coverslip. The entire cover slip area was examined using low power (10 X) then high power objectives (40 X).

3. Direct iodine wet preparation

Iodine wet preparation was made using Lugol's iodine solution (1%). A drop of iodine solution was placed on a glass slide using a piece of stick a small amount of feces was mixed with iodine and covered then by a glass cover slip. The preparation was examined under the microscope using low power (10 X) then high power objectives (40 X).

4. Direct eosin wet preparation

A drop of eosin solution placed on a glass slide. A small amount of stool was taken by a wooden stick and mixed with eosin. A cover slip was put gently on the preparation to avoid formation of air bubble. The preparation was examined under microscope.

Eosin does not stain *B. hominis* but provides a pink background which makes it easier to detect.

Formalin-ether concentration method:
Soon after direct smear microscopy, same fecal samples (collected from 1000 individuals) were concentrated by formalin-ether sedimentation technique as described by Cheesbrough.[10]

Results:
Prevalence of *B. hominis* in random population analyzed in rural residence is shown in Table 1. The prevalence rates vary from 12.50 to 38.23%. The highest positively was found in Mansurah locality (38.23%) and Quttah (31.25%). Out of 15 samples examined in Ashkeda, none was found positive for *B. hominis*.

Table 1: Prevalence of *B.hominis* among different rural localities of Wadi Al-Shati province.

Area	No. of Samples Examined			No. of Samples positive			Prevalence (%)
	Total	Male	Female	Total	Male	Female	
Ashkidah	15	9	6	0	0	0	00.00
Qirah	39	22	17	9	6	3	23.07
Brack	226	109	117	40	23	17	17.69
Zuwayah	42	27	15	6	6	0	14.28
Tamzawah	83	43	40	18	8	10	21.68
Ququm,	18	8	10	3	0	3	16.66
Aqar	54	18	36	9	4	5	16.66
Mahrugah	42	26	16	7	3	4	16.66
Al-Desa	61	12	49	17	6	11	27.86
Al- Qardah	78	34	44	13	5	8	16.66
Quttah	96	39	57	30	13	17	31.25
Bergen	38	18	20	6	2	4	15.78
Zahra	99	12	87	24	3	21	24.24
Wanzarik	50	32	18	13	8	5	26.00
Tamssan	8	2	6	1	0	1	12.50
Mansurah	34	11	23	13	6	7	38.23
Idri	17	5	12	3	2	1	17.64
Total	1000	427	573	212	95	117	21.20

The present date on prevalence rates are compared with other studies made on prevalence of *B.hominis* in stool samples in Libyan Arab Jamahiriya (Table 2).

Table 2: Prevalence of *Blasocystis hominis* among Libyans**.**

References	Locality	*B.hominis*(%)
Al-Fellani *et al*.(6)	Patients attending central laboratory in Sebha	18.55
Salem *et.al* (8)	Libyan patients in Sirt	29.6
Kassem *et.al* (11)	Children and neonates admitted to Iben-Sina Hospital in Sirt	12.57
Sadaga and Kassem (7)	School children in Derna	6.7
Saleh (9)	Patients attending central laboratory in Sebha	22.69
Khan *et al* (17)	Patients attending central laboratory in Sebha	26.61
Present study	**Community population in Wadi Al-Shati**	**21.20**

According to gender, 95 (22.24%) males and 117 (20.41%) females were found infected with *B. hominis* though this different was not statistically significantly (P>0.05).

All 1000 stool specimens were examined by direct wet mount microscopy using normal saline, Lugol's iodine, eosin stains and formalin-ether sedimentation technique for detection of *B. hominis*. Results are shown in Table 3.

Table 3: Comparison of methods for the detection of *B. hominis*

No. of stool samples	No. of samples positive by				P. value
	Direct smear microscopy			Concentration method	
	Saline	Iodine	Eosin	Formalin-ether sedimentation	
1000	183 (18.30)	197 (19.70)	196 (19.60)	212 (21.20)	**>0.05**

Figures in parentheses indicate percentages.

Iodine stained smears showed better contrast between central body and the cytoplasm**.** Concentration method yielded more numbers of vacuolar form of *B. hominis* compared to direct wet microscopy. Fecal concentration led to a higher positivity rate 21.20% compared to direct mount in normal saline 18.30%, iodine stain 19.70% and eosin stain 19.60%. Application of iodine and eosin stains mount smears does seem worthwhile in the detection of *B. hominis* compared to wet mounts in normal saline. No significant differen**ce** (P>0.05) was found using different direct wet mount and concentration method for the diagnosis of *B. hominis*. All stool samples found positive in direct wet mount microscopy, were also found positive by formalin-ether technique. Almost all of the *B. hominis* detected in direct smear and in concentration method were of the vacuolar form**.**

Discussion

In Libyan Arab Jamahiriya diagnosis of *B. hominis* is not routinely done in laboratories because laboratory technicians usually have low awareness about its diagnosis and association with human disease. For this reason, frequency of *Blastocystis* and source of infection are poorly known in Libya. In recent years studies have been made on incidence of *B. hominis*. The organism was reported in both symptomatic and asymptomatic individuals in Libya.[4,7,9,11] Shedding of *B. hominis* from these individuals could be source of infection to other in the region.

The present study is the first report on the prevalence of *B. hominis* in Wadi Al-Shati, South-West, Libya. Of 1000 stool samples examined, 212 (21.20%) were found positive for *B. hominis*. Almost similar prevalence rates have been described in other parts of the world, about 18% in Bethesda,[12] 25% in Jordan,[5] 25.78% in Venezuela,[13] 22.9% in Argentina[14] and 26.5% in Brazil.[15] In Libya indices of prevalence of *B. hominis* are 18.5%, 22.69% and 26.21% have been reported respectively among outpatients in city of Sebha.[4,6,9] Comparatively higher prevalence have been found abroad, 40.7% in Philippines[16] 36.9% in Thailand[17] and 32.0% in Pakistan[18] and 29.6% in Libya.[8] However, a lower prevalence of *B. hominis* has been recoded in other parts of the world. About 12% in Los Anglos,[19] 10% in Nepal,[20] 11% in New York,[21] 10.1% in Egypt,[22] 8.5% in Saudi Arabia[23] and 3.7% in China.[2] Moreover, lower positivity rate of *B. hominis* has been also reported in Libyan Arab Jamahiriya, 6.7% in Derna city and 12.57% in Sirt city[7,11] respectively.

In the present study, males were more infected than females with *B. hominis*. There was no significant difference between the prevalence rates of both sexes (P>0.05). These results are consistent with the findings of others, which described *B. hominis* infection, was more frequent in males than females, though the difference was not significant.[2,13,16,24,25] However, Khan *et al*.[26] reported significant differences of prevalence between males and females in the city of Sebha, Libya.

The transmission of *B. hominis* is generally assumed to be by fecal-oral route, in a manner similar to other gastrointestinal protozoa.[27] Water-borne transmission has been suggested.[17,25,28] Food-borne transmission of *B.*

hominis is also has been indicated.[29] The cyst form appears to be the principal infective form of *B. hominis*.[1,30,31]

Animals have been speculated to be a source of *B. hominis* transmitted to human because *Blastocystis* like organisms have been identified in a wild range of animals.[32-34] A higher risk of *B. hominis* infection was found in people with closed animal contact.[35] Recently molecular studies showed that *B. hominis* isolates from animals are closely related to isolates from humans.[36] These data support the possibility of zoonotic potential of *B. hominis*.[17,37]

The Libyan populations in Wadi Al-Shati zone are usually randomly assigned to various working units, agricultural practices, animal husbandry, private working units, and governments and thus transmission of *B. hominis* such as human to human, and animal to human may be taking place in this region. However, the study of Nimri and Batchoun[27] did not find a correlation between *B. hominis* infection and the presence of animals.

As a recent report suggested that cockroaches may act as vector for *Blastocystis* infection.[38] The rural localities have running water or toilets in their dwellings. In these cases, water collected outside in some localities may be play some role in the transmission of *B. hominis* in population. Cockroaches are plenty in rural localities of Wadi Al-Shati and may be capable of infecting some humans with this parasite.

The protozoan cysts and oocysts are relatively more resistant against an adverse environment.[39-41] Recently Mohammed *et al*.[4] reported that the infection of *B. hominis* was most common during summer and suggested that dry climatic conditions of Sebha favor the survival and transmission of the organism throughout the year in the population. Wadi Al-Shati province is neighborhood of Sebha city (about 70 Km away) and had same climatic conditions which favour the development, transmission and frequency of prevalence of *B. hominis* in different rural localities of this region.

In the present study, all the stool samples screened for *B. hominis* in direct wet smears in normal saline, iodine and eosin stains were also checked for the organism using formalin-ether sedimentation smears in iodine stain. The results showed that concentration method was found relatively more sensitive (21.20%) than direct wet mounts in normal saline (18.30%), iodine (19.70%) and eosin (19.60%) stains. Comparative analysis of the results, however, showed no significant difference between direct wet mounts in normal saline, iodine and eosin and formalin-ether sedimentation. Similar findings have been observed in a previous study.[15] In the present study, false-negative results were seen in smears prepared in normal saline compared to iodine stain which appears to give marked differentiation of parasite from background. A similar observation was reported using unstained preparation.[42] However, iron hematoxylin and trichome stains proved to be less sensitive than the other techniques for detection of *B. hominis*[15,43,44] respectively.

In the present study, formalin-ether sedimentation was found slightly more sensitive to *B. hominis* than direct smear microscopy of fresh stool specimens. These results are in accordance with Hussain Qadri *et al*.[45]; Adeen and Hale,[46]; Logar *et al*.[47] and Taamasri *et al*.,[28] who reported that formalin-ether method is beneficial compared to direct wet microscopy for the diagnosis of *B. hominis*. Guimaraes and Sogayar[48] suggested that spontaneous sedimentation concentration in normal saline is suitable for separating *B. hominis* from fecal material. Khan *et al*.[26] reported that sedimentation of fecal material in normal saline yields more positive results compared to direct microscopy. This increase in the detection efficacy of *B. hominis* in concentration methods, compared to direct microscopy, is probably due to presence of small numbers of *B. hominis* cells in the stool specimens of individuals, which are missed during routine direct smear microscopy. However, other authors have reported that concentration methods have no advantages over direct smear microscopy for detection of *B. hominis* in the stool samples.[3,45,49] They assumed that low detection efficacy appears due to necessary steps of shaking and centrifugation in formalin-ether technique that lead to rupture of *B. hominis* cells. Requena *et al*.[13] found that Willis concentration has no advantage over direct smear microscopy of fresh stool for detection of *B. hominis*.

References:

1. Stenzel D J and Boreham P L F.: Blastocystis hominis Revisited. Clin. J. Microbiol. 1996; 9(4): 563-584.
2. Wang K X, Licp, Wang J and Cui Y B.: Epidemiological survey of *Blastocystis hominis* in Huainan City, Anhui province, China. World. J. Gastroenterol. 2002; 8(5): 928-932.
3. Suresh K and Smith H.: Comparison of methods for detecting *Blastocystis hominis*. Eur. J. Microbiol. Infect. Dis. 2004; 23: 509-511.
4. Mohammed A A, Khan A H, Rugia M, Mabrok Z A and Farjani M.: Prevalence and clinical features of *Blastocystis hominis* among patients in Sebha, Libya. Sultan Qaboos Uni. Med. J. 2007; 7 (1):23-28.
5. Nimri L F: Evidence of an epidemic *Blastocystis hominis* infections in preschool children in northern Jordan. J. Clin. Microbiol. 1993; 31:2706-2708.
6. Al-Fellani M A, Abdullrahman O M, Khan A H and Abousaif A A.: Prevalence of intestinal parasites in Sebha, Libya. Garyounis. Med. J. 2005; 22: 56-60.
7. Sadaga G A and Kassem H H.: Prevalence of intestinal parasites among primary school children in Derna District, Libya. J. Egypt. Soc. Parasitol. 2007; 37(1): 205-214.
8. Salem R A A, Abdullah M E and Abdulgader A E.: Intestinal protozoa in Libyan patients in Sirt. Jamahiriya. Med. J. 2006; 6(1): 59-61.
9. Saleh M M.: Prevalence of human intestinal parasites in Sebha City. M. Sc. Thesis. Sebha University, Sebha, Libya, 2007.
10. Cheesbrough M.: District laboratory practice in tropical countries. 2nd ed. Cambridge university press. 2004: 191-199.
11. Kassem H H, Zead H A, and Sadaga G A.: Intestinal parasitic infection among children and neonatus admitted to Ibn-Sina hospital, Sirt, Libya. J. Eypt. Soc.Parasitol. 2007; 37(2): 371-380.
12. Zierdt C H, Swan J C and Hosseini J.: In-vitro response of *Blastocystis hominis* to antiprotozoal drugs. J. Protozool. 1983; 30: 332-334.
13. Requena I, Hernandez Y, Ramsay M, Salazar C and Devera R.: Prevalence of *Blastocystis hominis* among food handlers from Caroni municipality, Bolivar state, Venezuela. Cad. Saude. Pubika. 2003; 19: 1712-1727.
14. Minvielle M C, Pezzani B C, Cordoba M A, De Luca, Apezteguia M C and Basualdo J A.: Epidemiological survey of *Giardia* sp. *Blastocystis hominis* in an Argentinian rural community. Korean. J. Parasitol. 2004; 42: 121-127.
15. Nascimento S A and Moitinho M L R.: *Blastocystis hominis* and other intestinal parasites in a community of Pitaga City, Parana state. Brazil. Rev. Inst. Med.Trop. S. Paulo. 2005; 47(4):213-217.
16. Eleonor T B, Vincente Y B, Winifreda U D, Hyun H K and Dong C.: Infection status of intestinal parasites in children living in residential institutions in Metro Malino. The Philippines. Korean. J. Parasitol. 2004; 42: 67-70.
17. Leelayoova S, Rangsin R, Taamasri P, Naaglor T, Thathaisong U and Mungthin M.: Evidence of water-borne transmission of *Blastocystis hominis*. Am. J. Trop. Med. Hyg. 2004; 70: 658-662.
18. Yakoob J, Jafriw W, Jafri N, Khan R, Islam M, Beg M A and Zaman V.: Irritable bowel syndrome: In search of an etiology. Role of *Blastocystis hominis*. Am. J. Trop. Med. Hyg. 2004; 70: 383-385.
19. Garcia L S, Bruckner D A and Clancy M N.: Clinical relevance of *Blastocystis hominis*. Lancet. 1984; i: 1233-1234 (letter).
20. Babcock D, Houston R, Kumaki D and Shlim D.: *Blastocystis hominis* in Kathmandu, Nepal. N. Engl. J. Med. 1985; 313: 1419 (letter).
21. Sheehan D J, Raucher BG and McKitrick J C.: Association of *Blastocystis hominis* with signs and symptoms of human disease. J. Clin. Microbiol. 1986; 24: 548-550.
22. El- Shazly A M, Abdel Magied A A, El-Behbishi S N, El-Nahas H A, Fouad M A and Monib M S.: *Blastocystis hominis* among symptomatic individuals in Talkha Centre, Dakahlia Governate, Egypt. J. Egypt. Soc. Parasitol. 2005; 35: 653-666.
23. Khan Z A and Al-Khalife I S.: Prevalence of *Blastocystis hominis* among healthy food handlers in Dammam, Saudi Arabia. J. Egypt. Soc. Parasitol. 2005; 35: 395-401.
24. Sun T, Katz S, Tanenbaum B and Schenone C.: Questionable Clinical significance of *Blastocystis hominis* infection. Am. J. Gastroentrol. 1989; 84: 1543-1547.
25. Leder K, Hellord M E, Sinclair M I, Fairly C K and Wolfe R.: No correlation between clinical symptoms and *Blastocystis hominis* in immunocompetent individuals. J . Gastroenteral. Hepatal. 2005; 20: 1390-1394.

26. Khan A H, Al-Fellani M A, Rugaia A, Ali D and Omran A.: A comparsion of three different method for the diagnosis of *Blastocystis hominis*. Jamahriya. Med. J. 2008; 8(1): 31-34.
27. Nimri L F and Batchoun R.: Intestinal colonization of symptomatic and asymptomatic schoolchildren with *Blastocystis hominis*. J. Clin. Microbiol. 1994;32:2865-2866.
28. Taamasri P, Mungthin M, Raugsin R, Tongupprakarn B, Areekul W and Leelayoova S.: Transmission of *Blastocystis* related to the quality of drinking water. Southeast Asian. J. Trop. Med. Public Health. 2002; 31: 112-117.
29. Casemore D P.: Food-borne protozoal infection. Lancet, 1990; ii.1427-1432.
30. Singh M, Suresh K, Ho L C, Ng G C and Yap E H.: Elucidation of the life cycle of intestinal protozoan *Blastocystis hominis.* Parastiol. Res. 1995; 81: 446-450.
31. Vdovenko A A.: *Blastocystis hominis*: origin and significance of vacuolar and granular forms. Parasitol. Res. 2000; 86(1): 10.
32. Boreham P F L and Stenzel D J: *Blastocystis* in humans and animals:Morphology, biology and epizootiology. Adv Parasitol. 1993; 31:1-70.
33. Duda A, Stenzel D J and Boreham P F L.: Detection of *Blastocystis sp.* in domestic dogs and cats. Vet. Parasitol. 1998; 76(1-2): 9-17.
34. Abe N, Nagoshi M, Takami K, Sawano Y and Yoshikawa H.: A survey of *Blastocystis sp*. in livestock, pets and zoo animals in Japan. Vet. Parasitol. 2002; 106: 203-212.
35. Rajah S R H, Suresh K G, Vellayan S, Mak J W, Khairul A A, Init I, Vennila G D, Saminathan R and Ramakrishnan K.: *Blastocystis* in animal handlers. Parasitol. Res. 1999; 85(12): 1032-1033.
36. Noel C, Peyronnet C, Gerbod D, Edgcomb V P, Delgado-Viscogliosi P, Sogin M L, Capron M, Viscogliosi E and Zenner L.: Phylogenetic of *Blastocystis hominis* isolates from different hosts based on the comparsion of small-subunit rRNA gene sequences. Mol. Biochem. Parasitol. 2003; 126: 119-253.
37. Thathaisong U, Worapong J, Mungthin M, Tan-Ariya P, Viputtigul K, Noonai A and Leelayoova S.: *Blastocystis hominis* isolates from pig and horse are closely related to *Blastocystis hominis*. J. Clin. Microbiol. 2003; 41: 967-975.
38. Zaman V, Ng G C, Suresh K, Yap E H and Singh M.: Isolation of *Blastocystis hominis* from cockroach (Dictyptera: Blattidae). Parastiol. Rev. 1993; 79: 73-74.
39. Atias A.: Parasitologia Clinica, Ed. Puplicqiones. Tecincas. Mediterraneo. Santigo, Chile, 1993: 123-129.
40. Basualdo J A, Coto C E and DeTorres R A. (1996): Microbiologia biomedical. Ed. Atlante. Argentina.1996; 915-921: 956-963.
41. Soriano SV, Barbieri LM, Pierangeli NB, Giayetto AL, Manacorda AM, Castronovo E, Pezzani BC, Minvielle M and Basualdo JA.: Intestinal parasites and environment: Frequency of intestinal parasite in children of Nequen, Patagonia, Argentina. Rev. Latinoma. Microbiol. 2001; 43(2): 96-101.
42. Windsor J J, Macfaine L, Hughes-Thapa G, Jones S K, Whitside T M.: Incidence of *Blastocystis hominis* in feces samples submitted for routine microbiological analysis. Br. J. Biomed. Sci. 2002; 59(3): 154-157.
43.Amatoneto V, Rodriguez Alarcon R S, Gakiya E, et al.: Blastocystose: controverias e indenfinicoes. Rev. Soc. Bars. Med. Trop. 2003; 36: 515-517.
44. Tungtrongchitr A, Manatasathi S, Kositchaiwt C, Ongroichanakun J, Munkong N, Chinabutr P, Leelakusolvong S and Chaicampa W.: *Blastocystis hominis* infection in irritable bowel syndrome patients. Southeast Asian J. Trop. Med. Public Health. 2004; 35: 705-710.
45. Hussain Qadri S M, Al-Okaili G A and Al-Dayel F.: Clinical Significance of *Blastocystis hominis*. J. Clin. Microbiol. 1989; 27: 2407-2409.
46. Adeen W E and Hale D.: Use of Hemo-De to eliminate toxic agents used for concentration and trichome esaining of intestinal parasie. J. Clinl. Microbiol. 1992; 30: 1893-1895.
47. Logar J, Andlovic A and Poljsak-Prijatelj M: Incidence of *Blastocystis hominis* in patients with diarrhea. J. Infect. 1994; 28: 151-154.
48. Guimaraes S and Sogayar M I L.: *Blastocystis hominis* occurrence in children and staff members of municipal day-care enters from Botucatu, Sao Paulo State, Brazil. Mem. Inst. Oswaldo. Cruz Rio. J. 1993; 88: 427-429.
49. Leelayoova S, Wang J J, Chug L Y, Chang E E, Lai L C, Chen E R and Yen C M.: A survey on intestinal parasites of the school children in Kaohsiung Country. Kaohsiung. J. Med. Sci. 2002; 16: 452-458.