JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Pathogenicity and non-opportunistic character of *Blastocystis* spp.: a hospital-based survey in Central Cameroon

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Abstract

Introduction: *Blastocystis* spp. is a protist found in humans. Although usually the most frequent protozoa found in stool samples of both symptomatic and healthy subjects, its pathogenic or rather opportunistic role is yet to be clearly elucidated. To attempt to fill this gap, a cross-sectional study was conducted to compare the frequency of *Blastocystis* spp. in HIV positive (HIV^+) versus HIV negative (HIV^-) individuals in four health facilities of the Center Region of Cameroon.

Methodology: Stool samples were collected from 283 HIV positive and 245 HIV negative subjects and analyzed using direct diagnostic tests.

Results: A total of 46 (8.7%) individuals were found infected with *Blastocystis* spp., including 6.7% HIV positive and 11.0% HIV negative. This species was more frequent in urban and semi-urban areas than in rural areas, but evenly distributed among genders and age groups as well as among all sectors of activity. The prevalence of *Blastocystis* spp. (11.3%) was higher in HIV^+ patients with a CD4 count $\geq$ 500 cells / mm^3, but no significant difference was found among HIV clinical stages. Likewise prevalence, the mean number of cysts per gram of stool was similar between HIV positive and HIV negative individuals. People infected with *Blastocystis* spp. showed diverse clinical signs, but only flatulence was significantly more prevalent. The frequencies of these clinical signs were not related to HIV status.

Conclusion: No clear relationship links the infection with *Blastocystis* spp. to HIV, although its presence was associated with digestive disorder, suggesting that this parasite might not be opportunist.

Key words: *Blastocystis* spp.; prevalence; pathogenicity; HIV status.

J Infect Dev Ctries 2018; 12(5):373-379. doi:10.3855/jidc.10122

(Received 27 December 2017 – Accepted 18 March 2018)

Introduction

Blastocystis spp. is a protist found in humans and some animals [1-6]. Previous studies have shown a wider distribution in underdeveloped countries compared to developed ones [7-9]. Within the affected communities, this protozoan is highly prevalent amongst the poorest and most deprived individuals, exhibiting poor hygiene [6]. Although it is the most frequent protozoan reported in stool samples of both symptomatic and healthy individuals, its pathogenicity or clinical significance remains uncertain and controversial [4-7,10-11]. Indeed, *Blastocystis* spp. was considered as a commensal organism living in the human and animal intestine. The possibility that the immunosuppression influences the presence or severity of symptoms was proposed [12], though the presence of *Blastocystis* spp. in Human Immunodeficiency Virus (HIV) immunosuppressed individuals could not be confirmed [13].

The present study was an attempt to fill this gap by investigating the pathogenicity and the opportunistic character of *Blastocystis* spp. To this end, a cross-sectional study was conducted in the Central Cameroon in order to compare the frequency of this species between HIV positive (HIV^+) and HIV negative (HIV^-) individuals.

Methodology

Ethical consideration

The study was approved by the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. Prior sampling, authorizations were granted by the Directors of the health facilities (Yaoundé University Teaching

Hospital, Yaoundé Central Hospital, Ntui District Hospital and Akonolinga District Hospital) where the participants were enrolled. Verbal agreements were obtained from those who agree to participate, under the discretion of the leaders of health facilities. All participants were attributed a unique identifier and their data thus analyzed anonymously.

Study areas

This study was conducted in three divisions (Mfoundi, Mbam & Kim and Nyong & Mfoumou) of the Center Region of Cameroon. These divisions were chosen to represent different levels of urbanization. Indeed, the Mfoundi division is generally considered as an urban area (distances between buildings is less than 200m), although some of its peripheral quarters are rural settings. Ntui and Akonolinga, capitals of Mbam & Kim and Nyong & Mfoumou respectively, are semi-urban and semi-rural cities (intermediate zones), though the villages that are close to these cities are rural [14].

Study design

A hospital-based cross-sectional survey was carried out between July 2013 and September 2015 at the Yaoundé University Teaching Hospital (CHUY), the Yaoundé Central Hospital (HCY), the Ntui District Hospital and the Akonolinga District Hospital to compare *Blastocystis* spp prevalence between HIV infected and non-infected individuals. These health facilities were chosen since they have Units for management of HIV patients. People infected with HIV and visiting the management unit of each targeted health facility were eligible for the study and those who consented to participate in the study were enrolled as the reference group. HIV negative individuals were approached in the counselling services of the targeted health facilities after withdrawal of their HIV test results. Those HIV negative subjects who had already spent at least one year in the study area were eligible for the study and those who consented to participate were recruited and considered as the control group. Children under one year of age were excluded from the study due to the very low likelihood for exposure to contaminated water. All volunteers who agreed to participate in the study underwent a brief questionnaire and provided stool samples for *Blastocystis* spp. searching.

Questionnaire

All relevant information regarding HIV status, socio-demographic factors and clinical signs were gathered both by interview and consultation of medical notebooks of participants. The Cluster Differentiation 4 (CD4) cell counts were recorded from the medical registers of HIV positive patients. Indeed, CD4 counts were done every three months, according to the recommendations of the World Health Organization.

Sample collection and processing

A plastic vial was provided to each enrollee for stool sample collection. These vials were returned back with stools early in the morning the next day for analyses. Two approaches were used for stool sample processing:

Direct microscopy (wet mount)

The direct wet mounts of stools were performed using normal saline (0.9% NaCl solution), stained with Lugol's iodine (Art Pharmaceutical Ltd, Brossard, QC, Canada), and examined under light microscope (magnification 400x). *Blastocystis* spp. are spherical bodies of various sizes (4 - 15 µm of diameter) that seemed optically empty, with a large vacuole pushing to the periphery the cytoplasm that contains small refractive nuclei [4,15-16].

Formalin - Ether sedimentation

Stool samples provided by participants were weighted and 500 mg placed in a clean conical centrifuge tube containing 10 mL formalin (10%). In each conical centrifuge tube, the sample was dissolved and mixed thoroughly using a stick. The resulting suspension was filtered through a sieve into a beaker and the filtrate poured back into the same tube. The debris trapped by the sieve were discarded. Four milliliters of ether were then added to the mixture and homogenized by vigorous hand shaking. The content was therefore centrifuged at 2,000 rpm for 3 minutes. The supernatant was poured away and the sediments were stained with iodine. The preparation was then spread on a slide, covered with cover slip and examined using light microscope (magnification 400×) [17-19].

Statistical Analyses

All data relevant for this study were recorded into a purpose-built Microsoft Excel dataset and subsequently exported into PASW Statistics Version 18 (SPSS Inc., Chicago, IL, USA) for statistical analysis. Prevalence were expressed as the percentage of infected individuals among the total number of individuals examined. The mean number of *Blastocystis* spp. cysts was used to express the intensity of infection. The sampling fluctuation of intensity of infection was estimated using standard deviation (SD). Chi-square (χ^2), Fisher exact (f) and Mann-Whitney (U) tests were

used to compare prevalence and mean intensity of infection between different groups (HIV status, socio-demographic classes and clinical status). For all statistical analyses, the threshold for significance was set to 5%.

Results

Socio-demographic characteristics of the enrollees

Table 1 displays socio-demographic parameters of individuals enrolled in the study, both HIV negative and positive. A total of 528 individuals were enrolled, of which 283 (54%) were HIV positive and 245 (46%) were HIV negative. Among these enrollees, 294 were originated from urban settings, 162 from intermediate or semi-rural/semi-urban, and 72 from rural areas. Regarding gender, 172 (32.6%) males and 356 (67.4%) females were recorded. The sex ratio was female biased both among HIV positive patients (2.45) and seronegative subjects (1.72). According to the occupation of enrollees, 84% of participants were in the informal sector (small businesses and farmers), unemployed and inactive (children, pupils and students). The mean age of HIV positive subjects was 43.3 (SD: 10.5) years, with a minimum of 19 and a maximum of 68 years old, whereas the mean age in HIV negative participants was 30.3 (SD: 17.4) years old, varying between 1 and 80 years of age.

Prevalence and intensity of Blastocystis spp. infection

A total of 46 (8.7%) individuals were found infected with *Blastocystis* spp., including 19 (6.7%) HIV positive and 27 (11.0%) HIV negative subjects (Table 1). *Blastocystis* spp. was more frequent in urban (10.9%) and intermediate (7.4%) settings than in rural areas (2.8%). The difference observed between the prevalence of *Blastocystis* spp. was significantly higher among HIV^- compared to HIV^+ subjects in urban areas (P = 0.0156). Males (9.9%) and females (8.1%) were similarly infected with *Blastocystis* spp. Most of the age groups were infected, the frequencies being generally higher in seronegative than in HIV positive individuals. *Blastocystis* spp. was significantly more prevalent among HIV negative individuals aged 29 - 45 years (P = 0.0119). This parasite was found in all sectors of activity, HIV negative individuals involved in the formal sector being most affected than their HIV positive counterparts (P = 0.0116). As regard to intestinal antiparasitic treatment, the prevalence of *Blastocystis* spp. was significantly higher among HIV^- than in HIV^+ individuals who had taken an antiparasitic drug less than one month ago (P = 0.0333).

Table 1. Prevalence of *Blastocystis* spp. infection among HIV positive and HIV negative individuals according to socio-demographic parameters.

Socio-demographic parameters	HIV positives		HIV negatives		P-value
	N examined	N infected (%)	N examined	N infected (%)	
Areas	**283**	**19 (6.7)**	**245**	**27 (11.0)**	**0.0802**
Urban	202	16 (7.9)	92	16 (17.4)	0.0156*
Intermediate	39	3 (7.7)	123	9 (7.3)	1.0000
Rural	42	0 (0.0)	30	2 (6.7)	0.1702
Gender					
Male	82	7 (8.5)	90	10 (11.1)	0.5716
Female	201	12 (6.0)	155	17 (11.0)	0.0875
Age groups (years)					
[1-15]	0	ND	53	5 (9.4)	ND
]15-29]	30	0 (0.0)	80	4 (5.0)	0.2739
]29-45]	140	11 (7.9)	65	13 (20.0)	0.0119*
> 45	113	8 (7.1)	47	5 (10.6)	0.3223
Sectors of activity					
S_1	45	2 (4.4)	39	9 (23.1)	0.0116*
S_2	47	2 (4.3)	22	3 (13.6)	0.3175
S_3	170	14 (8.2)	94	9 (9.6)	0.7083
S_4	14	1 (7.1)	22	1 (4.5)	1.0000
S_5	7	0 (0.0)	68	5 (7.4)	1.0000
Last deworming date					
≤ 1 month	283	19 (6.7)	130	17 (13.1)	0.0333*
≥ 1 month	0	ND	115	10 (8.7)	ND

N: number; ND: not determined; *: Significant difference; S_1: employees of administrations, public and private enterprises (formal sector); S_2: agricultural workers or farmers (informal sector); S_3: non-agricultural workers or small businessmen (informal sector); S_4: unemployed; S_5: other inactive (children, pupils, students).

Table 2. Prevalence of *Blastocystis* spp. among HIV positive patients according to the immunity, treatment and clinical status.

Parameters	N	N infected (%)
CD4 count slices (cells/mm^3)		
< 200	32	0 (0.0)
[200-500[	154	8 (5.2)
≥ 500	97	11 (11.3)
P-value		*0.0453**
Clinical stages HIV		
A	12	0 (0.0)
B	243	19 (7.8)
C	28	0 (0.0)
P-value		*0.3275*
Antiretroviral		
Under ARV	240	19 (7.9)
Not under ARV	43	0 (0.0)
P-value		*0.0893*

CD4: cluster differentiation 4; HIV: human immunodeficiency virus; A, B, C: HIV clinical stages according to the Centers for Diseases Control and Prevention (CDC) classification (1999); N: number; *: Significant value; ARV: antiretroviral.

Table 3. Frequencies of clinical signs presented by *Blastocystis* spp. infected individuals during the last month prior sampling.

Groups	N	NW abdominal pain (%)	NW flatulence (%)	NW asthenia (%)	NW constipation (%)	NW diarrhea (%)	NW vomiting (%)	NW nausea (%)	NW blood in stool (%)	NW anorexia (%)	NW mucus in stool (%)
HIV-positives	19	15 (78.9)	10 (52.6)	6 (31.6)	3 (15.8)	4 (21.0)	2 (10.5)	2 (10.5)	2 (10.5)	0 (0.0%)	0 (0.0%)
HIV-negatives	27	25 (92.6)	13 (48.1)	4 (14.8)	4 (14.8)	2 (7.4)	0 (0.0%)	0 (0.0%)	5 (18.5)	1 (3.7)	3 (11.1)
***P*-value**		0.2133	0.5135	0.2771	1.0000	0.2133	0.1652	0.1652	0.6823	1.0000	0.2565

N: number of individuals infected with *Blastocyctis* spp; NW: number with.

Table 4 Frequencies of clinical signs presented by all individuals during the last month prior sampling.

***Blastocystis* spp**	N	NW Abdominal pain (%)	NW flatulence (%)	NW asthenia (%)	NW constipation (%)	NW diarrhea (%)	NW vomiting (%)	NW Nausea (%)	NW blood in stool (%)	NW anorexia (%)	NW mucus in stool (%)
Infected	46	40 (86.9)	23 (50.0)	10 (21.7)	7 (15.2)	6 (13.1)	2 (4.3)	2 (4.3)	7 (15.2)	1 (2.2)	3 (6.5)
Non Infected	482	372 (77.2)	96 (19.9)	269 (55.8)	149 (30.9)	56 (11.6)	26 (5.4)	78 (9.9)	79 (16.4)	120 (24.9)	21 (4.3)
***P*-value**		0.1259	0.0002*	< 0.0001*	0.0258*	0.7742	0.5517	0.0325*	0.8415	0.0005*	0.3489

N: number of persons examined; NW: Number with; *: Significant difference.

When taking into account the immunity and clinical status of HIV positive individuals, the prevalence of *Blastocystis* spp. was higher in the patients presenting with a CD4 count higher or equal to 500 cells / mm^3 (P = 0.0453) (Table 2). No significant difference was found in *Blastocystis* spp. prevalence as regard to HIV clinical stages and antiretroviral treatment.

Finally, no significant difference was found in mean *Blastocystis* spp. cyst counted in 500 mg stool of HIV seronegative (489.0; SD: 271.3) compared to HIV seropositive individuals (465.4; SD: 266.8) (U = 241.0, P = 0.7290).

Frequencies of clinical signs associated with the digestive disorder

The clinical signs recorded one month before sampling among individuals infected with *Blastocystis* spp. were abdominal pains (86.9%), flatulence (50%), asthenia (21.7%), constipation (15.2%), presence of blood in stool (15.2%), diarrhea (13.1%), presence of mucus in the stool (6.5%), vomiting (4.3%), nausea (4.3%) and anorexia (2.2%). No significant difference in frequencies of these clinical signs was found between HIV seronegative and HIV seropositive individuals (Table 3). *Blastocystis* spp. was found alone in 31 stool samples and in co-infection in 15 stool samples. The main organisms associated with *Blastocystis* spp. presence were yeasts (8 cases), *Entamoeba histolytica / E. dispar* (6 cases), *Pentatrichomonas intestinalis* (3 cases), *Iodamoeba buetschlii* (2 cases). When comparing the frequencies of clinical signs among *Blastocystis* spp. infected and uninfected individuals, only the flatulence was found associated with infection by this species (P = 0.0002) (Table 4).

Discussion

Blastocystis spp. is a protozoan known to dwell in the colon of its human host where it reproduces by binary division [1,20-25]. The objective of this study was to evaluate the pathogenicity and the opportunistic character of *Blastocystis* spp. among HIV negative and HIV positive individuals. Overall, 8.7% of individuals tested were infected with *Blastocystis* spp. The prevalence of *Blastocystis* spp. was not significantly different between HIV positive and HIV negative individuals (P = 0.0802). These results corroborate those of Trabelsi *et al*. [6] who reported a prevalence of 7.3% in Tunis, and those of Haileeyesus *et al*. [26] who observed a prevalence of 6.3% in HIV positive patients in Fitche in Ethiopia. Although *Blastocystis* spp. prevalence reported by Tian *et al*. [13] were relatively higher (13.9% in HIV^+ patients and 21.7% in HIV^- subjects) in Fuyang in China than in our study, the difference was not significant between the two groups (P = 0.1225).

Blastocystis spp. was more frequently observed in urban and intermediate areas than in rural areas. This parasite was evenly distributed among gender, age groups and was also found in people of all activity sectors, likely due to the cosmopolitan character of *Blastocystis* spp. [1,21-22].

Regarding HIV positive individuals, the prevalence of *Blastocystis* spp. was higher in patients presenting with a CD4 count ≥ 500 cells / mm^3. A significant difference in prevalence of infection with *Blastocystis* spp. was observed between CD4 slices (P = 0.0453) as previously reported by Tian *et al*. [13]. Indeed, these authors found an average CD4 count of 668 cells / mm^3 among HIV^+ subjects infected with *Blastocystis* spp. Furthermore, this parasite was recorded only in patients at the clinical stage B, and in people receiving antiretroviral, although no significant difference was found in prevalence of *Blastocystis* spp. between different HIV clinical stages, as well as when individuals were receiving antiretroviral therapy or not. These results, together with the fact that no significant difference was found in cysts' loads and HIV status, suggest that this organism has a non-opportunistic character [13].

People infected with *Blastocystis* spp. present, for most of them, clinical symptoms associated with digestive disorder. The frequencies of clinical signs were not associated with HIV status. The clinical signs observed were abdominal pain, flatulence, asthenia, constipation, presence of blood in stool, diarrhea, but only flatulence was found to be associated with infection by this species. Trabelsi *et al*. [6] found similar results as they had observed symptoms in 72.1% of individuals infected with *Blastocystis* spp. These results are slightly different from those reported by Bourré [27] who found symptomatic infection only in 38.7% of cases. Individual susceptibility likely associated with parasite strains having higher pathogenic characteristics may explain these differences [5,28]. Also, Tan and Suresh [25] suggested that the symptoms may be due to the accumulation of amoeboid forms of *Blastocystis* spp. strongly adhesive to the intestinal wall of the host. Lastly, co-infections were observed with yeasts, *Entamoeba histolytica, E. dispar*, *Pentatrichomonas intestinalis*, *Iodamoeba buetschlii* in the framework of the present study, the presence of these pathogens being likely amenable to induce potential bias related to the clinical disorder observed [6,20-24,29,30].

Limitations of the study

In the present study, *Blastocyctis* spp. was detected using only direct diagnostic tests of stool samples. This approach might have led to the underestimation of the prevalence of the disease since only microscopy was used to diagnose this parasitic species. Indeed, in vitro culture of *Blastocystis* spp. as well as nested Polymerase Chain Reaction (PCR) using DNA extracted from human stool samples have been described to generally display more positive cases than direct microscopy or standard PCR [31-32]. Also, the controversial results observed among different studies (including the present study) might be due to the presence of pathogenic and non-pathogenic genotypes. Unfortunately, the samples collected in the present study were not genotyped and this hypothesis could not be tested.

Conclusion

Blastocystis spp. is one of the most common human intestinal parasite. It was found in urban, semi-rural and rural settings in the Center Region of Cameroon. The presence of *Blastocystis* spp. was neither associated with HIV infection, nor with the clinical stage of HIV infection, suggesting that *Blastocystis* spp. might be a non-opportunistic parasitic species. Although the presence of this parasite was associated with digestive disorder, flatulence was the most important sign observed.

Acknowledgements

This study was supported by intramural funding of the Laboratory of Parasitology and Ecology of the University of Yaoundé I and the Laboratory of Parasitology of the Yaoundé University Teaching Hospital. The authors are thankful to all the individuals who consented to participate and contributed in this study. The authors declare that they have no competing interests.

Author's contributions

SJM conceived the study and designed the experiments, collected biological samples, performed laboratory experiment and drafted the manuscript. SF conceived the study, performed laboratory experiment, and statistical analyses, and helped to draft the manuscript. EB collected biological samples, performed laboratory experiment and drafted the manuscript. TTMT performed statistical analyses and helped to draft the manuscript. GAA helped to draft the manuscript. HCND performed statistical analyses and helped to draft the manuscript. ASE conceived and coordinated the study, designed the experiments and helped to draft the manuscript. FN conceived and coordinated the study, designed the experiments and drafted the manuscript. All authors read and approved the final manuscript.

References

1. Zierdt C, Rude W, Bull B (1967) Protozoan characteristics of *Blastocystis hominis*. Am J Clin Pathol 48: 495-501.
2. Chatelier N (2004) *Blastocystis* sp. in ruminant farming. Veterinary Medicine Thesis, Claude-Bernard University of Lyon I-France. 82 p. [Thesis in French].
3. Tan KS (2004) *Blastocystis* in humans and animals: new insights using modern methodologies. Vet Parasitol 126: 121–144.
4. Zierdt CH., Zierdt WS., Nagy B (1995) Enzyme-linked immunosorbent assay for detection of serum antibody to *B. hominis* in symptomatic infections. Parasitology 81: 127-129.
5. Lanuza M, Carbajal Ja, Villar J, Mir A, Borras R (1999) Soluble- protein and antigenic heterogeneity in axenic *Blastocystis hominis* isolates: pathogenic implications. Parasitol Res 85: 93-97.
6. Trabelsi S, Ichraf B H, Khaled S (2010) Epidemiological and clinical characteristics of *Blastocystis hominis*. Tunis Med 88: 190-192. [Article in French].
7. Amin OM (2002) Seasonal prevalence of intestinal parasites in the United States during 2000. Trop Med Hyg 66: 799-803.
8. Taamasri P, Leelayoova S, Rangsing R, Naaglor T, Ketupanya A (2002) Prevalence of *Blastocystis hominis* carriage in Thai army personnel based in Chonburi, Thailand. Mil Med 167: 643 – 646.
9. El Safadi D, Gaayeb L, Meloni D, Cian A, Poirier P, Wawrzyniak I (2014) Children of senegal river basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. BMC Infect Dis 14: 164.
10. Roberts T, Stark D, Harkness J, Ellis J (2014) Update on the pathogenic potential and treatment options for *Blastocystis sp*. Gut Pathog 6: 17.
11. Scanlan PD, Stensvold CR., Cotter PD (2015) Development and Application of a *Blastocystis* subtype-specific PCR assay reveals that mixed-subtype infections are common in a healthy human population. Appl Environ Microbiol 81: 4071-4076.
12. Garavelli PL., Scaglionel L, Rossi MR.., Bicocchi R, Libanore M (1989) Blastocystosis in Italy. Parasitol Hum Comp 64: 391-395.
13. Tian LG, Wang TP, Shan LV, Wang FF, Guo J, Yin XM, Cai YC, Dickey MK, Steinmann P and Chen JX (2013) HIV and intestinal parasite co-infections among a Chinese population: an immunological profile. Infect Dis Poverty 2: 18.
14. National Atlas of Physical Development of Cameroon (2013) Available: https://fr.slideshare.net/ninonjopkou/positionnement-gographique-des-activits-conomiques-du-cameroun. Accessed 16 May 2018.
15. Stenzel DJ, Boreham PF (1996) *Blastocystis hominis* revised. Clin Microbiol Rev 9: 563 – 584.
16. Suresh K., Smith HV, Tan TC (2005) Viable *Blastocystis* cysts in Scottish and Malaysian severage samples. Appl Environ Microbiol 71: 5619 – 5620.
17. Rousset JJ (1998) Practical copro-parasitology: interest and methodology, notions on parasites of the digestive tract, 1st Edition. Paris: ESTEM 106 p. [Book in French].
18. Ripert C (2003) Epidemiology of parasitic diseases: protozooses and helminthoses, reservoirs, vectors and transmission, 1st Edition. Cachan: Lavoisier 419 p. [Book in French].

19. Tuli L, Singh Deepak, Gulati Anil K, Sham Sundar, Tribhuban M Mohapatra (2010) A multiattribute utility evaluation of different methods for the detection of enteric protozoa causing diarrhea in AIDS patients. BMC Microbiol 10: 11.
20. Babb RR, Wagener S (1989) *Blastocystis hominis* – a potential intestinal pathogen. West J Med 151: 518 – 519.
21. Zierdt CH (1991) *Blastocystis hominis* – past and future. Clin Microbiol Rev 4: 61-79.
22. Debat Zoguereh D, Delmont J, Faugere B (1995) *Blastocystis hominis*, a potentially pathogenic parasite frequently identified after a tropical voyage. Press Medicine 24: 1578. [Article in French].
23. Jelinek T, Peyerl G, Löscher T, von Sonnenburg F, Nothdurft HD.(1997) The role of *Blastocystis hominis* as a possible intestinal pathogen in travellers. J Infect 35: 63 – 66.
24. Shlim D, Hoge C, Rajah R (1995) *Blastocystis hominis*, a cause of diarrhea in travelers? A prospective controlled study in Nepal. Clin Infect Dis 21: 97-101.
25. Tan TC., Suresh KG (2006) Amoeboid form of *Blastocystis hominis* - a detailed ultrastructural insight. Parasitol Res 99: 737-742.
26. Haileeyesus A, Teklu W, and Beyene P (2013) High prevalence of Diarrhoegenic intestinal parasite infections among non – ART HIV patients in Fitche Hospital, Ethiopia. PLoS One 8: e72634.
27. Bourré P (2007) *Blastocystis*: commensal or pathogenic? Study of 590 cases and review of the literature. Antibiotics 9: 20-24. [Article in French].
28. Dima ES, Meloni D, Poirier P, Osman M, Cian A, Gaayeb L, Wawrzyniak I, Delbac F, El Alaoui H, Delhaes L, Dei-Cas E, Mallat H, Dabboussi F, Hamze M, and Viscogliosi E (2013) Short report: Molecular epidemiology of *Blastocystis* in Lebanon and correlation between subtype 1 and gastrointestinal symptoms. Am J Trop Med Hyg 88: 1203.
29. Pinel C, Rejasse C, Picot S, Brenier- Pinchart M, Grillot R, Ambroise Thomas P (1999) *Blastocystis hominis*: epidemiological and clinical reflections on more than 3500 coprology tests. Ann Clin Biol 57: 601-604. [Article in French].
30. Sow D, Sylla K, Dieng T, Tine RC, Ndiaya M, Faye B, Ndiaye JL, Ndiaye D, Gaye O, Dieng Y (2015) Infection with *Blastocystis hominis* in Senegal: Epidemiological, clinical and parasitological aspects of diagnosed cases at CHNU de Fann in Dakar. Rev CAMES Health 3: 2424-7243. [Article in French].
31. Roberts T, Barratt J, Harkness J, Ellis J, Stark D (2011) Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis sp*. in clinical stool samples. Am J Trop Med Hyg 84: 308-312.
32. Santos HJ, Rivera WL (2013) Comparison of direct fecal smear microscopy, culture, and polymerase chain reaction for the detection of *Blastocystis sp*. in human stool samples. Asian Pac J Trop Med 6: 780-784.

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Conflict of interests: No conflict of interests is declared.