EVALUATION OF COMMON MICROSCOPIC TECHNIQUES FOR DETECTION OF *BLASTOCYSTIS HOMINIS*

By

WAAD T. ALDAHHASI[1], FAWZIA H. TOULAH[1] and MAJED H. WAKID[2,3*]

Department of Biology[1], Faculty of Science, University of Jeddah, Jeddah, and Department of Medical Laboratory Technology[2], 3Special Infectious Agent Unit, King Fahd Medical Research Center[3], Faculty of Applied Medical Sciences, P. O. Box 80324, King Abdul Aziz University, Jeddah 21589,, Saudi Arabia
(*Correspondence: E-mail: mwakid@kau.edu.sa, Fax: 009662-6952000 ext. 25502)

Abstract

Blastocystis hominis is the most prevalent eukaryotic intestinal protozoa that colonize the human gut, associated with intestinal disorders, with pathogenic controversial as most of them are asymptomatic. In this study, a comparison was carried out for sensitivity and specificity among two common using light microscopy, direct wet smear and Ritchie for the diagnosis *B. hominis* in human stools. Stool samples were collected from 29 Saudi and 111 non-Saudi patients, and examined for the detection of *B. hominis*. The infection was among age group between 31-50 years old and the highest rate was among male patients. Also, the results revealed that 98 samples were positive by direct smears; the sensitivity and specificity were 100% &75.9% respectively. Meanwhile, Ritchie technique revealed 86 positive samples, the sensitivity and specificity were 99% and 90.9% respectively.

Key-words: Saudi Arabia, *Blastocystis hominis,* parasites, direct smears, Ritchie.

Introduction

B. hominis is anaerobic protozoan eukaryotic parasite, which was firstly classified as yeasts. Relying on the sequencing of the gene encoding the 18S rRNA, *B. hominis* is now reclassified within the Stramenopiles group (Eme *et al*, 2017; Silberman *et al*, 1996). In human and a wide range of animals including birds, mammals and amphibians, nine subtypes of parasite were described (Andersen and Stensvold, 2016; Parkar *et al,* 2010). The prevalence ranged from 22% up to 100% in European, Asian & African Countries (AbuOdeh *et al*, 2016; Krogsgaard *et al*, 2015; El Safadi *et al*, 2014). The prevalence depends on the geographical areas, sanitation facilities level, personal hygiene and accuracy of diagnostic methods (Sánchez *et al*, 2017; El-Marhoumy *et al*, 2015; Stensvold, 2013; Abdulsalam *et al*, 2012). Diagnosis is based on microscopic examination of stool samples by using wet smears, iodine or trichrome, staining smears and formal ether concentration method to increase the microscopic examination performance, as well as *in-vitro* short-term culture. Now, the modern molecular biological techniques as real-Time PCR have provided more sensitive and specific methods for *B. hominis* diagnosis (Stensvold *et al*, 2007; Dogruman-Al *et al*, 2010; Zhang *et al*, 2012; Taghipour *et al*, 2018; Kok *et al*, 2019). Generally, the transmission is feco-oral route of cysts (Popruk *et al*, 2015). Infection can be asymptomatic or mild abdominal discomfort, constipation, diarrhea, nausea, bloating, anorexia, irritable bowel syndrome, dehydration, pruritis Tan *et al*, 2010; Nagel *et al*, 2016; Ajjampur and Tan, 2016).

This study aimed to compare sensitivity and specificity among the common microscopic methods for detection of *B. hominis.*

Materials and Methods

Samples collection: 140 stool samples of Saudi and non-Saudi patients were collected from different hospitals in Makkah. All stool samples were collected using clean sterile containers, then; samples were kept in laboratory fridge at 4°C till further examinations.

Microscopic direct smears examination: 1-2mg for each stool sample was mixed in a drop of normal saline NaCl; 0.9% (w/v) and Lugol's iodine on a microscope slide and each covered with a glass coverslip (22x22 mm), then examined by light microscope with 10x & 40x lenses (Amer *et al*, 2016).

Para-Pak® Trichrome stain (No. 400101): According to the manufacturer's instructions thin smears of polyvinyl alcohol (PVA)-preserved stool samples were prepared, left overnight to fully dry, stained for 6-8 minutes in Trichrome stain and then transferred into acidified ethanol for 10 seconds. After beginning of staining, slides were dipped immediately for twice quick dips in ethanol (95%), left for 5 minutes and finally in ethanol (96-100%) for 3 minutes. Each slide was placed in xylene for 3 minutes and mounted using Canada balsam in xylene, and then covered by coverslip. Slides were examined under oil immersion of light microscope.

Ritchie sedimentation technique: About 2 gm from each sample was emulsified in 10 ml of formal-saline (10%), and filtered via 2-3 gauze layers into a 15ml polypropylene tube. Tubes were centrifuged for 5 minutes at 2000 rpm. After discarding the supernatant, sediment was resuspended in a 10ml of 10% formal-saline, mixed thoroughly, then mixed with 3ml of diethyl ether and shake vigorously for 15 seconds then re-centrifuged at 2000rpm for 5 minutes. After centrifugation, the top of 3 layers were removed, and sediment was mixed with 2 drops of iodine. Microscopic examination was done by using 10x & 40x (Wakid, 2009).

Statistical analysis: Chi-square or P-value was used to test correlation between methods, stool consistency, nationality, ages & sex in relation to *B. hominis*. Kappa agreement was applied to validite diagnostic tests compared with the assumed gold standard test. Data were entered to the SPSS program (version 22). P value of <0.05 was defined as statistically significant.

Ethical approval was obtained from the Ministry of Health Makkah, Saudi Arabia.

Results

Out of 140 faecal samples, there were 98 infected cases. Six protozoan parasites were detected including 96 samples *B. hominis*, 8 *Giardia lamblia,* 6 *Endolimax nana,* 5 *Entamoeba coli*, 4 *Entam oeba histolytica*, and 1 *Iodamoeba butschlii*. Four helminthes were detected including 4 *Ascaris lumbricoides*, 3 for each of hookworms, and *Hymenolepis nana* and 2 *Trichuris trichiura*.

B. hominis infections were among Saudi (20.71%) and non-Saudi (79.29%) patients, with higher rate among workers from Bangladeshi (37.5%). There was significant relationships between nationality and ages with *B. homi-nis* using all techniques (P<0.05). Most patients were between 31-50 years old. A significant relationship was with six direct sme-ars (P<0.05). Stool samples were soft (77), loose (36), formed (21), hard (4), and watery (2) patients, without significant relationship between stool consistency and *B. hominis* infection by all techniques (P>0.05).

B. hominis in relation to used techniques: Out of 140 samples screened for *B. hominis*, direct smears detected 96 (68.6%) samples and Ritchie sedimentation 82 (58.6%).

B. hominis and other parasites in different nationalities showed no significant correlation between nationality and parasites other than *B. hominis* by all techniques (P>0.05). Also, there was no statistical difference in detection of all parasites including *B. hominis* by different techniques (P>0.05). There was a significant relationship between nationality and *B. hominis* infection using all techniques (P<0.001).

Parasites of 98 samples were 75.51%, 16.33%, 6.12%, 2.04% for single, double, triple and quadruple infection respectively.

Sensitivity and specificity: When direct smear was considered as a gold test, Ritchie technique showed 85.4% sensitivity & 100% specificity. Ritchie technique gave equal accuracy of 90% and diagnostic test agreement (Kappa test) 0.786. When Ritchie technique was considered as a nominated gold test, direct wet smear equal accuracy 90%, sensitivity 100%, specificity 75.9% and test agreement (Kappa test) 0.786.

The details were given in tables (1, 2, 3 & 4).

Table 1: Infection with *B. hominis* and other parasites in relation to nationality groups using all techniques

Parasite		Techniques	Non-Saudi (N =111) No. (%)	Saudi (N=29) No. (%)	Total (n=140) No. (%)	P-value [b]
Protozoa	*B. hominis*	Direct smears	89 (80.2)	7 (24.1)	96 (68.6)	<0.001
		Ritchie technique	78 (70.3)	4 (13.8)	82 (58.6)	<0.001
		P-value [a]	>0.05			
	E. histolytica	Direct smears	2 (1.8)	0 (0.0)	2 (1.4)	>0.05
		Ritchie technique	4 (3.6)	0 (0.0)	4 (2.9)	>0.05
		P-value [a]	>0.05			
	E. coli	Direct smears	4 (3.6)	1 (3.4)	5 (3.6)	>0.05
		Ritchie technique	4 (3.6)	1 (3.4)	5 (3.6)	>0.05
		P-value [a]	>0.05			
	E. nana	Direct smears	5 (4.5)	1 (3.4)	6 (4.3)	>0.05
		Ritchie technique	4 (3.6)	1 (3.4)	5 (3.6)	>0.05
		P-value [a]	>0.05			
	I. butschlii	Direct smears	1 (0.9)	0 (0.0)	1 (0.7)	>0.05
		Ritchie technique	0 (0.0)	0 (0.0)	0 (0.0)	-
		P-value [a]	>0.05			
	G. lamblia	Direct smears	8 (7.2)	0 (0.0)	8 (5.7)	>0.05
		Ritchie technique	7 (6.3)	0 (0.0)	7 (5.0)	>0.05
		P-value [a]	>0.05			
Helminths	Hookworm	Direct smears	1 (0.9)	0 (0.0)	1 (0.7)	>0.05
		Ritchie technique	3 (2.7)	0 (0.0)	3 (2.1)	>0.05
		P-value [a]	>0.05			
	T. trichiura	Direct smears	1 (0.9)	0 (0.0)	1 (0.7)	>0.05
		Ritchie technique	2 (1.8)	0 (0.0)	2 (1.4)	>0.05
		P-value [a]	>0.05			
	A. lumbri-coides	Direct smears	3 (2.7)	0 (0.0)	3 (2.1)	>0.05
		Ritchie technique	4 (3.6)	0 (0.0)	4 (2.9)	>0.05
		P-value [a]	>0.05			
	H. nana	Direct smears	1 (0.9)	0 (0.0)	1 (0.7)	>0.05
		Ritchie technique	3 (2.7)	0 (0.0)	3 (2.1)	>0.05
		P-value [a]	>0.05			

a: P-value in relation to type of technique, b: P-value in relation to nationality

Table 2: Types of infection and detected parasites using microscopy techniques.

Infection No. (%)	Parasites (+ve)	Nationality N.
Single 74 (75.51)	*B. hominis* (72)	Bangladeshi 29, Egyptian 1, Ghanaian 1, Indian 8 Indonesian 2, Jordanian 1, Moroccan 1, Pakistani 2, Palestinian 12, Filipinos 5, Saudi 6, Sudanese 1, Syrian 1, Vietnamese 1 &Yemeni 1
	A. lumbricoides (2)	Bangladeshi 2
Double 16 (16.33)	*B. hominis* + *E. nana* (4)	Bangladeshi 2, Egyptian 1, Pakistani 1
	B. hominis + *E. coli* (2)	Bangladeshi 1, Palestinian 1
	B. hominis + *A. lumbricoides* (1)	Palestinian 1
	B. hominis + hookworm (1)	Palestinian 1
	B. hominis + *H. nana* (1)	Indian 1
	B. hominis + *G. lamblia* (6)	Bangladeshi 3, Indian 1, Palestinian 2
	B. hominis + *E. histolytica* (1)	Indian 1
Triple 6 (6.12)	*B. hominis* + *E. nana* + *E. coli* (1)	Saudi 1
	B. hominis + *T. trichiura* +hookworm (1)	Sudanese 1
	B. hominis + *E. coli* + *E. histolytica* (1)	Palestinian 1
	B. hominis + *H. nana* + *G. lamblia* (2)	Filipinos 1, Pakistani 1
	B. hominis + *E. histolytica* + *I. butschlii* (1)	Pakistani 1
Quadruple 2 (2.04%)	*B. hominis* + *T. trichiura* + hookworm + *A. lumbricoides* (1)	Bangladeshi 1
	B. hominis + *E. nana* + *E. coli* + *E. histolytica* (1)	Indian 1
Total	98	

Table 3: Detection of *B. hominis* in relation to different categories using all techniques.

Categories		No.	Direct smears		Ritchie technique	
			No. (%) [a]	% [b]	No. (%) [a]	% [b]
Nationality	Bangladeshi	46	36 (37.5)	78.3	33 (40.2)	71.7
	Egyptian	2	2 (2.1)	100	2 (2.4)	100
	Ghanaian	1	1 (1.0)	100	1 (1.2)	100
	Indian	18	12 (12.5)	66.7	8 (9.8)	44.4
	Indonesian	3	2 (2.1)	66.7	1 (1.2)	33.3
	Jordanian	1	1(1.0)	100	1 (1.2)	100
	Moroccan	1	1 (1.0)	100	0 (0.0)	0.0
	Pakistani	5	5 (5.2)	100	4 (4.9)	80.0
	Palestinian	18	18 (18.8)	100	17 (20.7)	94.4
	Filipinos	8	6 (6.3)	75.0	6 (7.3)	75.0
	Saudi	29	7 (7.3)	24.1	4 (4.9)	13.8
	Sudanese	2	2 (2.1)	100	2 (2.4)	100
	Syrian	2	1 (1.0)	50.0	1 (1.2)	50.0
	Vietnamese	1	1 (1.0)	100	1 (1.2)	100
	Yemeni	3	1 (1.0)	33.3	1 (1.2)	33.3
	Total	140	96 (100)		82 (100)	
	χ^2		45.017		48.113	
	P-value		<0.001		<0.001	
Age groups	<30	35	35 (36.5)	66.0	30 (36.6)	56.6
	31-50	72	55 (57.3)	76.4	46 (56.1)	63.9
	>50	15	6 (6.3)	40.0	6 (7.3)	40.0
	Total	140	96 (100)		82 (100)	
	χ^2		7.881		3.056	
	P-value		0.019		0.217	
Sex	Female	35	19 (19.8)	54.3	16 (19.5)	45.7
	Male	105	77 (80.2)	73.3	66 (80.5)	62.9
	Total	140	96 (100)		82 (100)	
	χ^2		4.419		3.179	
	P-value		0.036		0.075	
Consistency	Formed	21	13 (13.5)	61.9	11 (13.4)	52.4
	Hard	4	2 (2.1)	50.0	2 (2.4)	50.0
	Loose	36	24 (25.0)	66.7	20 (24.4)	55.6
	Soft	77	55 (57.3)	71.4	47 (57.3)	61.0
	Watery	2	2 (2.1)	100	2 (2.4)	100
	Total	140	96 (100)		82 (100)	
	χ^2		2.342		2.196	
	P-value		0.673		0.7	

Table 4: Sensitivity, specificity and accuracy of diagnostic techniques

Techniques		P	N	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	Kappa Test	P-value
Direct smears*										
Ritchie	P	TP82	FP0	85.4	100	100	75.9	90	0.786 Substantial agreement	P<0.001
	N	FN14	TN44							
Ritchie*										
Direct smears	P	TP82	FP14	100	75.9	85.4	100	90	0.786 Substantial agreement	P<0.001
	N	FN0	TN44							

P: Positive N: Negative, FP: False positive TP: True positive, FN: False negative TN: True negative, *when considered gold standard method, PPV: positive predictive value NPV: negative predictive values

Discussion

In routine laboratory, the common techniques depend on microscopic examination using iodine Lugol's, sedimentation and trichrome staining (Adıyaman *et al*, 2015; van Lieshout and Roestenberg, 2015). In the present study trichrome stain was used with all protozoa positive cases. The consistency of stool samples ranged between formed to watery. Highest infection rate with *B. hominis* was in soft and loose stool (55% & 25.71%). This agreed with Das *et al*. (2013) in Bangladesh. In Iraq, patients' stools with *B. hominis* were liquid or semi-liquid stool played important role in case of persistence diarrhea in patients (Salman, 2015). This occurred

mainly with large numbers of this protozoan in stools (Al-Kaissi and Al Magdi, 2009).

Prevalence of *B. hominis* among males using direct smears and Ritchie were 73.3% & 62.9%, respectively. While in female patients were 54.3% & 45.7%, res- pectively. There was a significant difference when direct smears (P>0.05), was used. Nearly, the same data were obtained from patients in France and Egypt as the infection rate in males was higher than females (El Safadi *et al*, 2016; Hamdy *et al*, 2019). This may be due to the nature of males work in several fields ibut, females mainly work in houses.

The *B. hominis* highest infection rate was among patients aged from 31 to 50 years old. Also, patients aged 30 or below showed a higher infection rate than those above 50 years old. In Iran, *B. hominis* infection rate was more prevalent in ages between (40-49) years old (Khademvatan *et al*, 2018). In Iraq, *B. hominis* infection rates among patients with gastrointestinal disorders were high in elder age group (61 to 80) years old (Hammood *et al*, 2016). In Brazil, affected children aged 5-9 years (Segui *et al*, 2018). This discrepancy in *B. hominis* infection among different ages referred to outcome of the different risk or hazardous activities among each group (Mohammad *et al*, 2017).

In the present study, the single infection was detected in 74 samples (72 with *B. hominis* and 2 with *A. lumbricoides*) and *B. hominis* co-infection of with other parasites was in 24 samples. Pagheh *et al.* (2018) in Iran showed the co-infection of *B. hominis* with other intestinal parasites mainly protozoa).

Generally, the present results showed that protozoan infections (85.71%) were higher than helminthic ones (8.57%). Similar result was reported (Barbosa *et al*, 2018; Oishi *et al*, 2019). This may be due to the direct mode of intestinal protozoan transmission. Also, their life cycle is simpler than the helminthic complicated ones that need one or more intermediate host taking much time to form infective stage (Maizels *et al*, 1993).

The present study results revealed that the

foreigner workers with highest intestinal parasites rates were Asians, mainly from Bangladesh, Palestine and India. A study on migrant workers in Malaysia found, the majority of infected workers were from Indonesia: 43.3%, Nepal: 20.9%, Bangladesh: 18%, India: 12.1% & Myanmar: 5.9.2% (Sahimin *et al*, 2016). In Al-Baha, higher intestinal parasites were among Indonesians (41.42%), Indians (22.89%), Bangladeshis (10.93%), Filipinos (8.89%), Pakistanis (9.71%), Sri-Lankans (5.09), then Egyptians and Syrians (2.03%) (Mohammad and Koshak, 2011). In Al-Khobar, intestinal parasites were more among workers Sri Lankans (44.8%), Indonesians (28%), Filipinos (18%) and then Indians (8.6%) (Abahussain and Abahussain, 2005). Also, in Sharjah, the highest infection rates were among Indian workers (30.4%), Bangladeshis (20.6%), Pakistanis (16.1%) and Afghan (11.9%) (Dafalla *et al*, 2017). Infection was *B. hominis* (68.6%) followed by *G. lamblia* (5.7%) then *E. histolytica* (2.9%), which agreed with (Pestehchian *et al*, 2015) in Iran.

In the present study, high *G. lamblia* was among Bangladeshis (37.5%), but, *E. histolytica* was among Indians (50%) and *E. coli* among Palestinians (40%). A study in India, showed the following infection rates: *G. lamblia* (69.5%), and *E. histolytica* (15.7%) (Yogyata and Binita, 2011). Taha *et al.* (2013) in Al-Madinah reported that the protozoa infection was 21.9% with *G. lamblia* and 17.8% with *E. histolytica/ E. coli*. *E. histolytica* among Sudanese workers was 37.1% and for Pakistani ones, the commonest was *G. lamblia* (34.14%). Imam *et al.* (2015) in Al-Madinah detected *E. histolytica* in 19 cases (27.5%), *G. lamblia* in 13 cases (18.8%), and *E. coli* in 5 cases (7.2%).

In the present study, *E. nana* was the common protozoa (4.3%), followed by *E. coli* (3.6%), & *I. butschlii* (0.7%). In Riyadh City, the protozoa were *E. coli*, *I. butschlii* & *E. nana* (4.08%, 1.79% & 1.75%) respectively (Eligail *et al*, 2010). But, total non-pathogenic parasites were similar as being

transmitted by oral fecal contamination, co-infected with pathogens (Poulsen and Stensvold, 2016).

Among non-Saudis (Bangladeshi & Palestinian workers) were *A. lumbricoides* with highest infection rate (2.8%), *T. trichiura* i (1.4%) was among a Sudanese and similarly in a Bangladeshi worker. Hookworm was found in a Palestinian, a Sudanese, and a Bangladeshi worker. But, *H. nana* was found in an Indians, a Filipino and a Pakistani worker. Al-Madinah study showed that *T. trichiura* was the commonest worms among those from Philippines, Sri Lanka and Indonesia with rate of 38.5%, 33.3% & 31.8% respectively, followed by *A. lumbricoides* in Filipinos & Indonesians (30.76% & 23.1%). None of Bangladeshi workers was infected with helminthes. In Al-Madinah, the high rate was among Ethiopians followed by Indians and then Sri Lankans. In Ethiopians hookworm was 15.8% and *T. trichiura* and *A. lumbricoides* were lower (0.3% & 7.9%), respectively (Imam *et al*, 2015).

The non-Saudi patients were from Asian (75%) and African countries (4.28%). Most of them were infected with *B. hominis* (68.6% & 58.6%) by using direct smears and Ritchie technique respectively. Same findings were reported in Makkah during Hajj, as most of foreign workers were from Asia (Wakid, 2009). Also, in Makkah *B. hominis* was (59.8%) among Asians (Ahmed *et al*, 2015). In Qatar Asian workers showed 87.6% *B. hominis* rate (Behnke, 2013), but 68.6%, 67.6% were from Western and Eastern Asia respectively (Abu-Madi *et al*, 2015). Lu and Sung (2009) in Taiwan found that *B. hominis* was among immigrant Indonesians, Vietnamese, Filipinos & Chine se (26.4% 20.6%, 19.3%& 7.6%) respectively.

By microscopy, *B. hominis* was 68.6%. Bangladeshis showed the high infection rate (37.5%), but one was among Ghanaians, Jordanians, Moroccans, Syrians, Vietnamese and Yemenis (1% each). Ritchie's data were lower than direct smears (58.6%). Ritchie technique is used mainly to detect cysts, ova

and larvae, but not trophozoites (Srichaipon *et al*, 2019). Destruction or distortion of parasites during Ritchie's preparation gave false results (Wakid *et al*, 2009). Using Ritchie technique as gold standard method, microscopy sensitivity was 100% & specificity was 75.9%. In Iraq, microscopy sensitivity was (74.14%) & specificity was 100% (Uobeed *et al*, 2015). Using direct smears as gold standard method, sensitivity and specificity for Ritchie technique were 85.4% & 100%, respectively.

Conclusions

Periodically examination for intestinal parasites is a must for hand-workers by different diagnostic methods. Comparing microscopic and molecular techniques to detect *B. hominis* is ongoing and will be published in due time. To authors' knowledge none used direct smears as a gold standard method.

Acknowledgements

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