Prevalence of *Blastocystis hominis* and *Giardia lamblia* Parasites in Patients of Four Regions in East – South Baghdad

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Summary

A total of 250 stool specimens from patients live in Al-Ameen, Al-Mashtal, Al-Baladiyat and Al-Nahrawan, districts of Baghdad city were collected. The watery and loose specimens were wet mounted with normal saline and buffered methylene blue then all specimens concentrated and examined with Lugol's iodine solution. The protozoal parasites of medical importance *Blastocystis hominis* and *Giardia lamblia* were respectively detected in 59 (24.6%) and 42 (17.5%), samples with mixed infection were in eight samples. Differences due to district lacked significance. Statistical significance was observed when the influence of age category was tested ($P<0.05$). The highest infestation percentage with both parasites was noticed in the 5 – 10 years category whereas the lowest percentage was in the 19 – above years category. Seasonal variations and sex of the infected patients with both protozoa did not reach statistical significance. Significant differences were observed between number of infected patients with *B. hominis* and *G. lamblia* in the age categories of respectively Al-Ameen and Al-Baladiyat districts. Significance was recorded in the differences between percentages of the age categories' females as well as of the age category 5 – 10 years males and females that were infected with *G. lamblia* Similar significance marked the percentages of infection of both sexes with *B hominis* in the age categories

Keywords: ***Blastocystis hominis, Giardia lamblia,* south-east Baghdad,**

إنتشار الطفيلي *Blastocystis hominis* و الطفيلي *Giardia lamblia* في مرضى من أربعة مناطق جنوب شرق بغداد

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الخلاصة

جمعت 250 عينة غائط من مرضى يقطنون في مناطق الأمين ، المشتل ، البلديات و النهروان من بغداد حضرت شرائح رطبة باستخدام المحلول الملحي و صبغة المثلين الأزرق المتعادلة مباشرة عند استلام العينات ذات القوام المائي و غير المتماسك، الجزء المتبقي من هذه العينات و العينات ذات القوام اللين خضعت للترسيب لأعداد شرائح رطبة استخدمت فيها صبغة Lugol's iodine من الأوالي الطفيلية ذات الأهمية الطبية ، وجد الطفيلي *B hominis* في 59 عينة (24.6%) و الطفيلي *G lamblia* في 42 عينة (17.5%). ثمانية عينات فيها إصابة مزدوجة لم تؤثر المنطقة السكنية معنويا على نسب

الإصابة بالطفيليين (P<0.05) كان تأثير الفئة العمرية معنويا على نسب الإصابة بالطفيليين. و سجلت أعلى نسبتي إصابة في الفئة العمرية 5 – 10 سنوات و أوطأ نسبتي إصابة في الفئة العمرية 19 سنة فما فوق لم يكن لفصول السنة و الجنس تأثيرا معنويا على عدد المصابين بالطفيليين لوحظت المعنوية الإحصائية في عدد الإصابات بطفيلي *B hominis* في الفئات العمرية لمنطقة الأمين و في عدد الإصابات بطفيلي *G lamblia* في الفئات العمرية لمنطقة البلديات وجدت المعنوية الإحصائية في فروق نسب إناث المجاميع العمرية المصابة بالطفيلي *G lamblia* و في الفروق بين الجنسين المصابين بهذا الطفيلي من الفئة العمرية 5-10 سنوات. تميزت الإصابة بالطفيلي *B hominis* بوجود تأثير معنوي للفئات العمرية على نسب الإصابة في كلا الجنسين.

Introduction

Blastocystis hominis and *Giardia lamblia* are worldwide enteropathogens parasites of human being and animals These anaerobic zoonotic protozoa have been detected in fecal samples of patients including travelers complaining from diarrhea (1, 2 and 3) *Bhominis* early in the last century was classified as a yeast within the genus *Schizosaccharomyces* then classified in the phylum Protozoa subphylum Sporozoa (4). Then Zierdt (5) put forward evidence for classification of this enteroparasite within the phylum Protozoa subphylum Sarcodina. It has multiple morphological forms (6) pointed out to a vacuolar vacuolar multivacuolar ameboid granular and cystic forms. Boreham and Stenzel (7) described vacuolar as the most common form beside ameboid granular and encysted forms in the large intestines of the host. The encystaion takes place before leaving the host (7). Ingestion of the cyst is followed by excystation and in case the active form succeeds in establishing an inflammation diarrhea abdominal pain fever loss of appetite and weight nausea vomiting dizziness and flatulence are expected symptoms (8,9,10 and 11). Clinical observations have associated *B. hominis* with irritable bowel syndrome and emphasized the necessity for detection and diagnosis of the pathogenic intestinal protozoa (12). This zoonotic parasite has also been associated with inflammatory bowel disease (11) ulcerative colitis (13) and adenocarcinoma of the colon and rectum (14).

Giardia lamblia (synonymous: *G. duodenalis G. intestinalis*) is an anaerobic flagellated binucleated parasitic protozoa from Mastigophora class habitat in duodenum down to the upper part of the ileum in man and wide variety of vertebrates It is a main cause of traveler's diarrhea Infection with this parasite giardiasis is manifested with symptoms include abdominal cramps nausea flatulence and acute or chronic diarrhea (15, 16 and 17).

It is one of the most prevalent pathogenic protozoa and is estimated to result in over 200 million human infections per year (18). Malabsorption syndrome and steatorrhoea are also noticed in giardiasis (19). Infected children showed depressed rate of growth (stunting) in both symptomatic or asymptomatic disease (20) beside mental retardation (21) Giardiasis in children (1 – 5 years old) significantly decreased serum levels of zinc and iron (22). It has been

blamed for cases of irritable bowel syndrome (12) gall bladder cancer (23) and duodenal villous atrophy (24). It has two forms the active (trophozoite) and the encysted (1,25 and 26). It is very interesting to know that the inventor of the microscope Antonie van Leeuwenhoek examined his own diarrheal stool in 1681 and observed the *G. lamblia* trophozoite (27). The morphological features of this parasite forms are commonly used in the inexpensive routine laboratory diagnosis Kits for antigen detection methods such as direct fluorescent immunoassay enzyme immunoassay and rapid dipstick-like tests have been made available (28). Furthermore an advanced molecular technique polymerase chain reaction has also been employed (29). Various investigations have been carried out to determine the prevalence of *B hominis* and *G lamblia* in Baghdad (30) reported 23.5% for *B hominis* and 19.9% for *G lamblia* while (31) found 60% for *B hominis* and 12.2% for *G lamblia.*

The present investigation aims are identification of *B hominis* and *G lamblia* forms in fecal samples of patients suffering from intestinal ailments including diarrhea Prevalence of the two pathogens was presented according to the involved regions of Baghdad city age groups seasons and sex of the infected patients.

Materials and methods

During the period between June 2009 and May 2010, 240 patients were referred by internal medicine specialists to a private clinical laboratory for stool examinations including those specific for protozoa The patients were residents of four regions of Baghdad city (Al-Ameen, Al-Mashtal, Al-Baladiyat and Al-Nahrawan). They complained from gastrointestinal disorders and the symptoms covered diarrhea abdominal pain cramp nausea loss of weight and sometimes vomiting. Immediately after receiving the watery and loose stool specimens physiological saline solution and buffered methylene blue were employed to obtain wet stained slide preparations (32). Formalin (10 %) was added to the remaining portions of these stool specimens and to the soft stool specimens The formalin-added specimens were further handled in the Parasitology Department Faculty of Veterinary Medicine They were subjected to the modified Ritchie concentration method to have wet slide preparations stained with Lugol's iodine solution (33). The stained preparations were closely examined under a binocular light microscope looking for the protozoa *Blastocystis hominis* and *Giardia lamblia*. For determination of the statistical significance Pearson Chi-Square Test was employed in analysis of the recorded data (34) using the specified program of Microsoft Office Excel 2007 (Microsoft Corporation USA).

Results

The consistency of the stool specimens ranged between foamy-watery loose and soft Microscopic examination of the stained stool preparations confirmed the infection of 59 out of the 240 patients (24.6 %) with *B hominis* The protozoan *G lamblia* has established itself in stool specimens of 42 out of the 240 patients (17.5 %) Percentages of infection cases with *B hominis* in Al-Ameen, Al-Mashtal, Al-Baladiyat and Al-Nahrawan regions were respectively 75%, 79%, 46% and 46% whereas those with *G lamblia* were 50%, 46%, 50% and 29% respectively Mixed infection with the two parasites was confirmed in eight patients. The region did not significantly influence the infection percentages (Table 1). Figure 1 shows number of the male and female patients infected with *B. hominis* or *G. lamblia*. Thirty one out of 125 males (24.8%) and 28 out of 115 females (24.3%) were infected with *B hominis*. On the other hand infection with *G lamblia* was observed in 19 out of 125 males (15.2%) and 23 out of 115 females (20%) Differences due to sex of the infected patients failed to reach statistical significance.

Table (1): Number and percentage of patients infected with *G lamblia* and *B hominis* within the four regions†

Region	Total Number of Patients	Number of Infected Patients (%)	
		With *G lamblia*	With *B hominis*
Al-Ameen	82	12 (50)	18 (75)
Al-Mashtal	63	11 (46)	19 (79)
Al-Baladiyat	53	12 (50)	11 (46)
Al-Nahrawan	42	07 (29)	11 (46)
Total	240	42 (175)	59 (246)

† Region lacked significant (p > 005) influence on percentage of patients infected with the protozoa

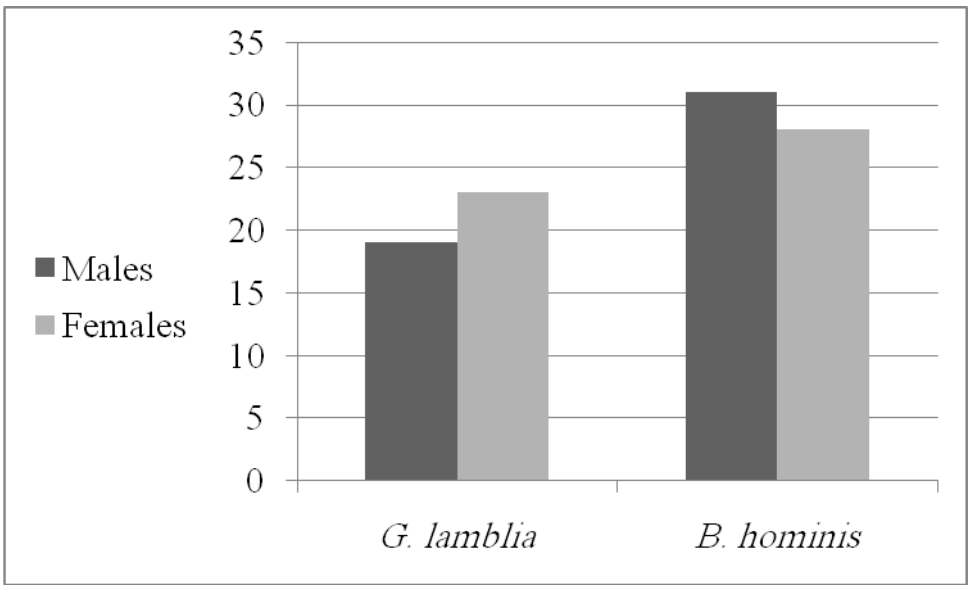

Figure (1): Number of male and female patients infected with *G lamblia* and *B hominis* Differences due to sex statistically not significant (p > 005)

Total number of patients and number of infected patients are tabulated within four age categories (Table 2) Age category played a significant role in the observed differences between percentages of infection with the particular parasite ($p < 0.05$) The age category 5 – 10 years showed the highest number of patients infected with *B. hominis* (29; 121%) and *G. lamblia* (22; 92%) Lowest numbers were observed for *B. hominis* (6; 25%) and *G. lamblia* (3; 13%) within the age category 19 – above years.

Table (2): Number and percentage of patients infected with *G lamblia* and *B hominis* within four categories of age†

Age Category (years)	Total Number of Patients	Number of Infected Patients (%)	
		With *G lamblia*	With *B hominis*
< 1 – 4	64	13 (54)	16 (67)
5 – 10	65	22 (92)	29 (121)
11 – 18	45	04 (17)	08 (33)
19 – over	66	03 (13)	06 (25)
Total	240	42 (175)	59 (246)

† Age category had a significant ($p < 0.05$) effect on infection percentage with the parasites

Season did not demonstrate any effect on the number of patients whose stool specimens were positive for presence of each parasitic protozoan (Table 3). The highest percentage of infection with *B hominis* was recorded during summer (24; 10%) followed by autumn (13; 54%) spring (12; 50%) and winter (10; 42%). Summer was also the season during which highest incidence of infection with *G lamblia* (16; 67%) The number of infected cases during Spring reached 13 (54%) followed by Autumn (8; 33%) and Winter (5; 21%).

Table (3): Number and percentage of patients infected with *G. lamblia* and *B. hominis* within the seasons†

Season	Total Number of Patients	Number of Infected Patients (%)	
		With *G lamblia*	With *B hominis*
Summer	67	16 (67)	24 (10)
Autumn	53	08 (33)	13 (54)
Winter	56	05 (21)	10 (42)
Spring	64	13 (54)	12 (50)
Total	240	42 (175)	59 (246)

† Differences between percentages of infection with the two parasites were not significant (p > 005)

Total numbers of male and female patients as well as number of male and female patients infected with *B. hominis* or *G. lamblia* within each of the four regions are listed in Table 4. Statistical analysis revealed a non-significant effect of sex within the regions on the number of infected patients. The highest number of male patients infected with *B hominis* was 10 out of 33 (30.3%) within Al-Mashtal region whereas the number of female patients was nine out of 30 (30%) within Al-Mashtal and nine out of 37 (24.3%) within Al-Ameen region. Within Al-Baladiyat region the number of males infected with *G lamblia* was as high as eight out of 26 (30.8%). The respective number of females was 8 out of 30 (26.7%) within Al-Mashtal region.

Table (4): Total number and number of male and female patients infected with *G lamblia* and *B hominis* within the four regions†

Region	Total Number of Patients		Number of Infected Patients (%)			
			With *G lamblia*		With *B hominis*	
	Male	Female	Male	Female	Male	Female
Al-Ameen	45	37	5 (111)	7 (189)	9 (200)	9 (243)
Al-Mashtal	33	30	3 (091)	8 (267)	10 (303)	9 (303)
Al-Baladiyat	26	27	8 (307)	4 (148)	6 (231)	5 (185)
Al-Nahrawan	21	21	3 (143)	4 (190)	6 (286)	5 (238)
Total	125	115	19	23	31	28

† Number of male and female patients infected with both protozoa out of the total number of patients within each region did not show significant (p > 005) differences

Table 5 presents total number of patients and number of patients infected with the parasites within the four regions. Statistically significant impact for age category on infection was recorded in Al-Ameen region for *B hominis* and in Al-Baladiyat region for *G lamblia* (P< 0.05). The observed differences between percentages of the males and females infected with *B hominis* within age

categories reached statistical significance (P<0.05) as presented in Table 6 Significant differences were detected between females of the age groups infected with *G lamblia* Percentages of the males and females infected with *G lamblia* within age group 5 – 10 years also showed significant differences**.**

Table (6): Total number and number and percentage of male and female patients infected with *G lamblia* and *B hominis* within the four age categories

Age Category (years)	Total Number of Patients		Number of Infected Patients (%)			
			With *G lamblia*		With *B hominis*	
	Male	Female	Male	Female†	Male†	Female†
< 1 – 4	40	24	9 (225)	4 (167)	9 (225)	7 (292)
5 – 10	32	33	7 (219)†	15 (455)†	15 (469)	14 (424)
11 – 18	20	25	1 (50)	3 (120)	4 (200)	4 (160)
19 – over	33	33	2 (61)	1 (30)	3 (91)	3 (91)
Total	125	115	19	23	31	28

† Significant (p < 005) differences between females of the age categories infected with *G lamblia* between males and females of the age category 5 – 10 years infected with *G lamblia* and between males and females of the age categories infected with *B hominis*

Discussion

Based on the morphological aspects results of the microscopic examination confirmed presence of *B hominis* and *G lamblia* in some but not all of the patients fecal samples. The point that should be emphasized here is that the fecal samples were obtained from symptomatic cases In another word randomized sampling was not part of the experimental design. Prevalence of *B hominis* (24.6%) in all of the patients' samples was close to 23.5% (30) and 21.36% (35) that were observed in randomized samples in Baghdad city However a low percentage (06%) was also reported in Baghdad (31). In Basrah city the prevalence of this parasite was 36% in patients suffering from sickle-cell anemia (36). In a Turkish university hospital (37) found 12.2% of *B hominis* and (38) found 19.8% in rural area of Antakya while in Hamedan west of Iran 21% were reported (39) *B hominis* was detected in 52.7% of immigrants in the city of Naples southern of Italy (40). Blastocystosis in humans is more prevalent in developing countries 30% - 50% than in developed countries 15% to 10% (41). Detection of *G lamblia* in 17.5% of the patients' stool specimens went along with 18% in Babylon Province (42) 19.94% in Baghdad (30). 15.39% in Lebanon(43) and 16.5% in Turkey(38) Higher percentages have been recorded 217% in Baghdad (44). 38.5% in Dohuk Province (45) and 37.7% in Riyadh Saudi Arabia (46). On the other hand low rates of incidence 45% in southern Italy (40) 74% (47) and 12.2% (31) in Baghdad have been encountered. The differences could be attributed to several factors such as sampling socio-economic education and sanitation Results of Table -1 also showed that infection rates with *B hominis* and *G lamblia* in the four regions were

comparable Mixed infection with both parasites is not uncommon (12, 48 and 49). Sex of the patient did not significantly influence the infection rate with the parasites (Figure 1 and Table 4). This result agreed with that found by (46, 50 and 38). However, a report indicated that higher rates of infection occurred in males (44) possibly due to unrestricted outdoor activities of the males. The significant differences between infection rates due to sex within the specified age groups (Table 6) could be attributed to the social circumstances under which the sampled population was subjected.

Age category (Table 2) had a significant effect Polluted outdoor environment insufficient education contacting infected subjects and improper toilet and food hygiene are possible predisposing factors (51and 52) Significant impact of age on incidence of infection with these intestinal protozoa was noticed in age ranges of 1 – 3 (50), 1 – 5 (47), 5 – 9 (44), 6 – 7 (53), and 1 – 10 (31). The observed significant role played by age category within the residency region (Table 5) might add the importance of the sanitary measurements. The results has pointed out to the high rate of infection during summer time (Table 3) but the rate was close to those of other seasons. This finding reflects the fact that Baghdad if not most provinces of the country has a long hot dry summer and short moderately cold winter Hot and dry weather encourages parasitic infection (54). Higher rates of infection during summer have also recorded in the Palestinian city of Nablus (55). With such background on the drastic consequences of infection with these intestinal pathogenic protozoa there is a real necessity for periodic exploration of their prevalence.

The fluctuation in rates of infection incidence definitely reflects the extents of water food and soil contamination as well as the maintenance services of the sewage system and other sanitary measurements. Hygienic education should not be overlooked.

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