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Diet Profile in Asymptomatic University Students of FACSA Cohort Colonized by Blastocystis: Brief Report

Janeth Oliva Guangorena-Gómez[1]*, Claudia Muñoz-Yáñez[1]

[1]Universidad Juárez del Estado de Durango, Facultad de Ciencias de la Salud Sixto Ugalde y Palmas I S/N Col Revolución, Gómez Palacio, Durango, México

***Correspondence:** Janeth Oliva Guangorena-Gómez, Universidad Juárez del Estado de Durango, Facultad de Ciencias de la Salud Sixto Ugalde y Palmas I S/N Col Revolución, Gómez Palacio, Durango, México, C.P. 35050. Tel 8717886642

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Key words: *Blastocystis*; fiber; legumes; red meats; Subtypes; 24hr reminder

1. Abstract

Blastocystis is a protozoan present in the intestine of healthy and symptomatic humans, the prevalence of infection has been estimated at up to 100%; its transmission is mainly by the faecal-oral route through contaminated water or food. Due to the direct effects on the intestinal level and gut microbiota, the implications of this infection for human health have been described. On the other hand, diet is essential in modifying gut microbiota. We determined the occurrence of *Blastocystis*, and the most frequent subtypes, and their association with the diet of university students. A cross-sectional study was conducted to analyze faecal samples of university students of FACSA cohort. The students answered two 24-hour dietary recalls (weekdays and weekend days) once they signed the informed consent. Associations between qualitative and quantitative variables were made with comparisons between medians of two groups. Of 139 students, the frequency of *Blastocystis* carriers was 48.9%. *Blastocystis* was associated with lower BMI, and lower consumption of red meat, ST3 is associated with lower consumption of red meat and dairy products and higher consumption of legumes, and ST4 with a more balanced diet in terms of protein and carbohydrate consumption. The diet of subjects carriers of *Blastocystis* subtypes 3 and 4 suggests the association with healthy intestinal microbiota.

2. Introduction

Blastocystis is a unicellular protist that colonizes the large intestine of humans and a wide variety of animals [1,2]. This protist is transmitted by the faecal-oral route through contaminated water or food and close contact with animals [3]. Children, the elderly, and immunocompromised persons appear highly susceptible to *Blastocystis* invasion [4].

Blastocystis is one of the most frequent eukaryotes; its prevalence is up to 100% [5]. Its clinical importance is not evident because there are controversial results in various studies; it has been related to a higher prevalence in healthy individuals [1] since they harbour a greater diversity of intestinal bacteria and a healthy intestinal microbiota [6] , also it has been increasingly considered a member of the healthy intestinal microbiota [7] and is even postulated in research as a potential probiotic agent [8]; whilst other studies suggest its association with Irritable Bowel Syndrome and intestinal dysbiosis [9,10]. This disparity is believed to be due to the wide variety of existent subtypes [11]. Previous studies have associated different pathologies with particular subtypes; subtype four (ST4) has been associated with diarrhoea. In Spain, 94% of identified patients with diarrhoea were monoinfected with *Blastocystis* [12]; in Denmark, patients with acute diarrhoea were associated with this subtype [13]; as well as Italian patients with IBS and diarrhoea [14]. The prevalence of the ST4 subtype has been reported as low (7.5%) in asymptomatic Spanish populations in Northern Spain [15] and in Madrid [16,17], suggesting that ST4 may be more virulent than other *Blastocystis* STs. In recent *in vitro* studies, the analysis of *Blastocystis* ST4 and the intestinal microbiota interaction showed that co-incubation with *Blastocystis* ST4 had a beneficial influence on most intestinal bacteria. At the same time, ST4 significantly inhibited the growth of *Bacteroides vulgatus*, a common pathogen in the genus *Bacteroides* [18]. In addition, Leing Deing et al., in a mouse colitis model, found *Blastocystis* ST4 colonization promoted T helper 2 (Th2) response defined by interleukin (IL)-5 and IL-13 cytokine production, and T regulatory (Treg) induction from colonic lamina propria in normal healthy mice [18]. Furthermore, faecal microbiota transplantation of *Blastocystis* ST4-altered gut microbiome to colitis mice reduced the severity of colitis, which was associated with increased production of short-chain fat acids (SCFAs) and anti-inflammatory cytokine IL-10 [19].

On the other hand, it is known that diet is an essential modulator of the intestinal microbiota; cumulative epidemiological data suggest a protective effect of high intakes of dietary fibres (the primary source of SCFAs) for maintaining a healthy body weight [20]. As previously mentioned, one of the metabolic roles of gut microbiota is to harvest energy from the host diet [21]; also, there is still controversial if *Blastocystis* induces the diversity and richness of the gut microbiota or if the profile of a healthy diet promotes *Blastocystis* colonization. Therefore, this study aimed to characterize the diet of clinically healthy university students colonized by *Blastocystis*.

3. Subjects And Methods

3.1 Sample collection

The study has a cross-sectional design performed on the FACSA Cohort previously reported [22].

3.2 Measurement of diet

Anthropometric characteristics of height and weight were recorded by a nutritionist. The students were questioned about their physical activity to proceed with the online application of two 24 hours dietary records, one on weekdays and one on weekend days which analyzes the average daily intake of food and nutrients [23].

3.3 Blastocystis identification

3.4 Parasitological examination

Samples were collected in containers with 10% formaldehyde for coproparasitological exams in triplicate. Each microscopic identification of *Blastocystis* was carried out on a different deposition day. The sample preparation was developed as described previously [24].

3.5 Determination of *Blastocystis* by Polymerase chain reaction (PCR).

DNA extraction was performed according to the manufacturer's instructions previously described [24] .

3.6 Subtyping of *Blastocystis*

For Blastocystis genotyping, a set of sequence-tagged site primers derived from products of randomly amplified polymorphic DNA (RAPD) previously described [25] sequences were used [26,27].

3.7 Statistical analysis

A descriptive analysis of the variables studied of the 139 participants was performed. The Kolmogorov-Smirnov test evaluated the normality of the continuous numerical variables. The quantitative variables were summarized in terms of means and standard deviation o the median and interquartile range (IR25-IR75), and the qualitative variables were summarized in frequencies and proportions. Non-parametric U-Mann-Whitney test was used for comparisons between the medians of the two groups. A chi-square test (x^2) or Fisher's exact test were applied for the bivariate analysis of qualitative variables. A *P* value <0.05 was considered significant. The statistical analysis was performed using the Stata® Statistics Package, version 13.0 and GraphPad Prism 9.4.

4. Results

4.1 General characteristics of study participants

In this study, *Blastocystis* was detected in 68/139 (48.92%) samples by microscopical examinations and polymerase chain reaction (PCR). The median age and interquartile ranges were 20 (18-21) years and 20 (19-21) years for *Blastocystis*-negative and -positive individuals, respectively. The median weight and interquartile range among *Blastocystis*-negative and *Blastocystis*-positive individuals were 67.8 (57.4 -86.4) kg and 63.5 (53.55-73.55) kg, respectively (U-Mann-Whitney-Wilcoxon test *P*=0.02). The percentage of females and males participants was 71.22 % (99/139) and 28.78% (40/139), respectively. Subjects colonized by *Blastocystis* have a lower body mass index than non-colonized subjects (U-Mann-Whitney-Wilcoxon test, *P*=0.024), Table 1.

4.2 Key characteristics according to the colonization of *Blastocystis* and diet.

Diet does not seem to be related to the presence of *Blastocystis*. Still, subjects colonized with *Blastocystis* consume more fibre than non-colonized subjects 12.7 (7.9-21) vs. 10 (6-16.5) (U-Mann Whitney test, *P*=0.116) and consume less amount (g) and proportion (%) of protein 75.5 (51-116) vs 90.5 (70.5-128) and 16.34 (12.31-21.11) vs 17.28 (13.43-23.11) U-Mann-Whitney test, (*P*=0.05), it should be noted that when consumption was compared in terms of food groups between those not colonized and regarding subtypes, subtype 4 is associated with a balanced consumption of proteins 14.45% (11.26-18.56) compared to those not colonized by this subtype 17.28% (13.13-22.46) (U of Man-Whitney-Wilcoxon test *P*=0.04) (Tables 2 and 3). Also, this subtype is associated with low BMI, *P*=0.024 (Table 1). Subtype 3 was the most frequent, 27.94% 19/68 (data no showed), and is related to higher consumption of legumes and less consumption of red meat and dairy products (Figures 1, 2 and 3).

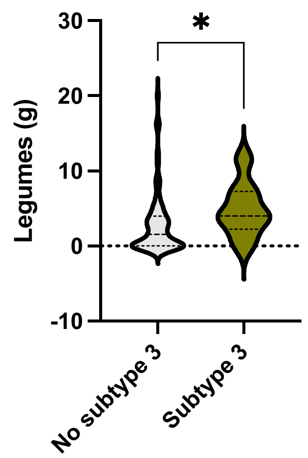

Figure 1. Consumption of legumes in non-carriers and carriers of *Blastocystis* Subtype 3. Subjects not colonized by Subtype 3 consume fewer legumes than subjects carrying Subtype 3. U-Mann-Whitney-Wilcoxon test. (*P*= 0.046)

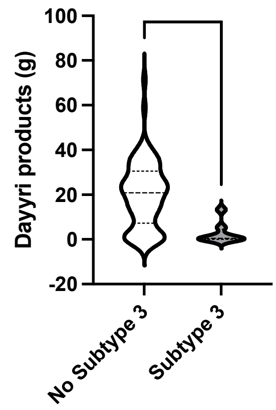

Figure 2. Dairy consumption in non-carriers and carriers of *Blastocystis* Subtype 3. Subtype 3 non-carriers consume more dairy than Subtype 3 carriers. U-Mann-Whitney-Wilcoxon test. (*P*= 0.028).

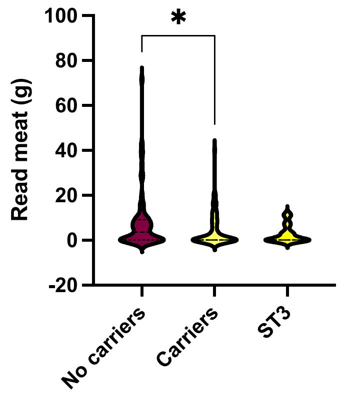

Figure 3. Consumption of red meat in non-carriers and carriers of *Blastocystis* and Subtype 3. Subjects not colonized by *Blastocystis* consume more red meat than carriers of *Blastocystis* (*P*=0.014) and carriers of Subtype 3 (*P*=0.10).

Table 1: Demographic data and BMI of participants among *Blastocystis*-no carriers and *Blastocystis*-carriers groups.

	Total	No Carriers	Carriers	*P* value
Woman, n (%)	99(71.22)	51 (51.52)	48 (48.88)	0.871
Man, n (%)	40 (28.78)	20 (50.00)	20 (50.00)	
Age, median IR (Years)	20 (18-21)	20 (18-21)	20 (19-21)	0.083
Weight, median IR (kg)	65.6 (55.5-78.7)	**67.8 (57.4-86.4)**	**63.65 (53.55-73.55)**	**0.025**
BMI, (Kg/m^2)	23.83 (20.64-27.52)	**24.29 (21.8-29.2)**	**23.12 (20.26-26.60)**	**0.024**

M: Median; IR: Interquartile range; g: grams; U of Mann-Whitney-Wilcoxon test; t-student test; kcal:kilocalories; BMI: body mass index; *P*<0.05.

Table 2: Nutritional variables were evaluated based on the analysis of 24-hour recalls in two *Blastocystis*-no carriers and *Blastocystis*-carriers groups.

Macronutrients	**Total**	**No carriers**	**Carriers**
Energy Intake (kcal)	2017.5 (1441.2-2532.2)	2047.9 (1560-2545.6)	1924.0 (1421-2525)
Fiber (g)	11.6 (7-18)	10 (6-16.5)	12.7 (7.9-21)
Carbohydrates (g)	242.5 (174-329)	254 (185-307)	222 (169-341)
Carbohydrates (%)	48.42 ± 13.34	49.22 ± 15.26	49.65 ± 10.97
Protein (g)	83 (64-119)	90.5 (70.5-128)	75.5 (51-116)
Protein (%)	16.98 (12.89-21.33)	17.28 (13.43-23.11)	16.34 (12.31-21.11)
Total lipids (g)	74 (47-105)	78 (46-110)	68 (49-96)
Total lipids (%)	33.35 ± 11.30	32.94 ± 11.96	33.80 ± 10.61
Saturated fatty acids (g)	22.5 (16-35)	22.5 (13.5-34)	22.5 (18-35)
Monounsaturated fatty acids (g)	22.5 (14-37)	23.5 (13.5-37)	22 (17-39)
Polyunsaturated fatty acids (g)	12.0 (7-21)	12 (7-21)	13.5 (8-22)
Cholesterol (mg)	242.1 (143.4-384.8)	221.3 (139.9-398.4)	253.9 (144.8-384.6)
Food groups			
Oils and fats (g)	0.76 (0-2.4)	0.83 (0-2.1)	0.32 (0-2.5)
Animal source foods (g)	13.6 (7.3-21)	14.3 (7.3-23.6)	12.40 (7.28-19.7)
Read meat (g)	**5.99 ± 10.4**	**7.76 ± 12.60**	**4.14 ± 7.20**
Dairy products (g)	13.6 (1.1-30.6)	13.6 (0-32.9)	14.9 (1.6-30.2)
Cereals (g)	23.3 (15.0-34)	21.8 (14.3-35.5)	24.1 (16.1-31.9)
Legumes (g)	3.0 ± 7.9	1.84 ± 3.7	4.30 ± 10.54
Oil seeds (g)	0.16 ± 0.85	0.09 ± 0.54	0.23 ± 1.08
Fruits (g)	8.4 ±11.85	6.77 ± 9.50	10.15 ± 13.76
Vegetables (g)	2.7 (0-7.7)	2.81 (0-7.7)	2.63 (0-7.7)
Fruits and vegetables (g)	4.8 (0.6-11.4)	4.54 (0.5-9.70)	5.76 (1.05-11.75)

g: grams; %: percentage; mg: milligrams; ±: standard deviation; U of Mann-Whitney-Wilcoxon test kcal: kilocalories; $P<0.05$

Table 3: Analysis by subtypes 1,2,3,4, and 7 of *Blastocystis* regarding the consumption of fiber, carbohydrates, proteins, and total lipids.

	Fiber (g)	**Carbohydrates (%)**	**Proteins (%)**	**Total Lipids (%)**
ST1				
No carriers	11 (7-18)	48.50 ± 13.68	16.83 (12.94-21.33)	33.27 ±11.44
Carriers	14 (11.25-14.46)	47.42 ± 8.33	19.64 (11.17-21.33)	34.34 ± 9.8
ST2				
No carriers	11.25 (7-18)	48.54 ± 13.30	16.41 (12.89-21.33)	33.35 ± 11.32
Carriers	12 (8-32)	43.83 ± 17.05	22.46 (20.42-26.69)	33.4 ±13.13
ST3				
No carriers	11 (6-18)	48.28 ± 13.41	17.28 (12.94-22.2)	33.36 ±11.21
Carriers	14 (11.25-18)	49.41 ± 13.23	16.41 (12.17-21.33)	33.29 ± 12.36
ST4				
No carriers	11 (6-16.22)	48.12 ± 13.84	**17.28 (13.13-22.46)**	33.31 11.51
Carriers	16.31 (11-22)	50.71 ± 8.59	**14.45 (11.26-18.56)**	33.63 + 9.89
ST7				
No carriers	11.62 (7-17.5)	48.23 ± 13.59	16.98 (12.89-21.22)	33.57 ± 11.42
Carriers	11.74 (7-21)	52.09 ± 6.44	17.35 (13.24-24.99)	29.12 ± 8.39

g: grams; %: percentage; ±: standard deviation; ST1: Subtype 1; ST2: Subtype2; ST3: Subtype 3; ST4: Subtype 4; ST7: Subtype 7.

5. Discussion

In this cross-sectional study of 139 clinically healthy university students, *Blastocystis* was associated with lower BMI in colonized subjects; These findings agree with Beghini et al. (2017) [28]. Also, consistent with findings from the study of the Danish subjects [29]. Between specific subtypes, only ST4 reached statistical significance ($P = 0.03$ between average weight and obesity). Besides, Tito et al. (2019) found a positive and significant correlation ($R = 0.26$ $P = 0.00028$) between ST4 and *Akkermansia* and *Methanobrevibacter* [30,31].

5.1 Analysis by food groups and subtypes

It's known that ST3-*Blastocystis*-carriers have a high abundance of *Prevotella*, *Methanobrevibacter*, and *Ruminococcus*, while those *Blastocystis*-free subjects have a high percentage of *Bacteroides* [32]. Asnicar F. et al. (2021) reported exciting findings of the presence of *Prevotella copri* and *Blastocystis* as markers of improved postprandial glucose response; both were strongly linked with favourable glucose homeostasis and a decrease of the estimated visceral adipose tissue mass [33]. This association between Blastocystis and glucose homeostasis may be due to the consumption of legumes since subjects colonized by subtype 3 consume less red meat. This group of patients can meet their nutritional needs with legumes due to the high amount of protein, fibre, iron, folate, and potassium [38]; furthermore, legumes have low-fat content (in almost all cases), as well as low sodium and glycemic index, absence of cholesterol and gluten [39], which could influence the association with Blastocystis. Additionally, legumes, such as chickpeas, lentils, and beans, are rich in fibre resistant to digestion that increases the production of short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate, which are produced by gut microbial fermentation [22]. Recent evidence suggests that dietary fibre and gut microbial-derived SCFAs exert multiple beneficial effects on the host´s energy metabolism by improving the intestinal environment [34], also promoting the secretion of the intestinal mucosa, and reducing intestinal permeability. Additionally, the fibre in legumes promotes beneficial bacteria growth and activity due to its prebiotic effect [35]. It should be emphasized that legumes have contributed significantly to human nutrition.

Regarding subtype 3, it was related to less consumption of red meat (Figure 3), although it was not significant (P=0.10). However, high consumption of red meat can favour the proliferation of proteolytic bacteria and cause intestinal dysbiosis; consistent with this finding, Stensvold et al. found that individuals not carrying *Blastocystis* contain more proteolytic bacteria, which may be due to the consumption of red meat [7]. Meat is not only a significant source of valuable proteins but also of vitamins such as A, B1, B12, and niacin, iron, zinc, and other micronutrients [36]. Recent evidence from cohort studies and meta-analyses of epidemiological studies indicates that the long-term consumption of increasing amounts of red meat, mainly processed meat, is associated with an increased risk of total mortality, cardiovascular disease, colorectal cancer, and type 2 diabetes in both men and women [37]. The commensal microorganism members of the gut microbiota, such as *Blastocystis*, are essential for living beings, although their clinical importance is still debated. Various factors can intervene in its proper development, such as diet; however, also lifestyle, antibiotic consumption, or the ageing process [38] will determine changes in its composition and, as a consequence, the possibility of suffering from diseases [39]. However, at present, sufficient scientific evidence reinforces the importance of diet for the establishment, structure, and functional activity of the intestinal microbiota [40]. In conclusion, this study showed that nutritional status was negatively associated with the consumption of red meat and the presence of *Blastocystis*; also, the diet of subject carriers of *Blastocystis* ST3 was richer in legumes, and less consumption of dairy products and ST4 was associated with less BMI and a more balanced diet. However, metagenomic and metabolomic studies must be carried out.

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7. Conflict Of Interest

The authors declare no conflict of interest

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