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Research paper

Blastocystis subtypes isolated from travelers and non-travelers from the north of Poland – A single center study

Monika Rudzińska[a,*], Beata Kowalewska[a], Piotr Wąż[b], Katarzyna Sikorska[a], Beata Szostakowska[a]

[a] Department of Tropical Medicine and Epidemiology, Medical University of Gdańsk, Poland
[b] Department of Nuclear Medicine, Medical University of Gdańsk, Poland

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ABSTRACT

Blastocystis is a common, enteric protist of humans and animals with a worldwide distribution and unclear clinical relevance. Nine out of 17 genetically diverse subtypes occur in humans. We analysed the distribution of *Blastocystis* subtypes and the intensity of invasion in relation to the gastrointestinal tract disorders and travels to different continents. 122 *Blastocystis* stool cultures were subtyped via polymerase chain reaction (PCR) with seven pairs of subtype-specific, sequence-tagged-site (STS) primers. Five subtypes of *Blastocystis* were detected: ST3 (59%), ST2 (19.7%), ST1 (13.1%), ST6 (3.3%), ST7 (3.3%), and two mixed infections with ST1/ST3 (1.6%). ST1 was detected exclusively in travelers to hot climate zones and ST2 was found more frequently in people visiting other continents compared to those who never left Poland. We found no correlation between gastrointestinal tract disorders, *Blastocystis* STs, and parasite load. There was no age predisposition to the *Blastocystis* infection. We established the distribution of *Blastocystis* STs among Poles traveling to different continents and never leaving Poland. Our study sheds more light on the problem of importing *Blastocystis* infection. It shows that certain subtypes detected in Europe can be imported due to travel or migration. Collecting data on the travel history of the surveyed persons is necessary to clarify this matter.

1. Introduction

Blastocystis is a unicellular, anaerobic, eukaryotic protist occurring in the intestines of humans and a variety of animals, including primates, mammals, birds, reptiles, amphibians and even insects (Wawrzyniak et al., 2013; Stensvold and Clark, 2016). Its prevalence in humans ranges from 2.5% to 56% in developed countries and up to 100% in developing countries and differs within geographical regions, countries, and communities (Yoshikawa et al., 2004; Scanlan et al., 2014; El Safadi et al., 2014; Seyer et al., 2017). This divergence is primarily explained by the varying levels of sanitation infrastructure and hygiene habits, as well as by the diversity of the studied populations such as age group (children/adults), health status (symptomatic/asymptomatic), residence (village/town) (Yoshikawa et al., 2004; Javanmard et al., 2018)

Until 2007, the name *Blastocystis hominis* was used because it was firmly believed that *Blastocystis* in humans was a different species than those occurring in animals. Further genetic diversity studies have led to the identification of 17 subtypes of *Blastocystis* and have shown that some subtypes can occur both in humans and animals (Wawrzyniak et al., 2013). This led to changes in the nomenclature: *Blastocystis hominis* was replaced by *Blastocystis*, followed by a subtype number (e.g., ST1) (Stensvold et al., 2007). Ten STs (ST1-ST9, ST12) were identified in humans, but the majority of human infections (90–95%) are caused by ST1-ST4 (Clark et al., 2013; Ramírez et al., 2016; Forsell et al., 2017).

Subtypes (morphologically indistinguishable) identified in humans are also found in mammals: pets, livestock, wilds, and birds (except for ST9 that was detected so far only in people). High similarity or even identity of DNA sequences of *Blastocystis* STs from humans and animals suggest the potential of zoonotic transmission (Thathaisong et al., 2003; Tanizaki et al., 2005; Fathy, 2011; Wang et al., 2014; Wang et al., 2018). The most likely way of infection is a faecal-oral route through contaminated water or food (Leelayoova et al., 2008; Fathy, 2011; Plutzer and Karanis, 2016).

The pathogenicity of *Blastocystis* has not been unequivocally proven. Many researchers have point out a wide range of clinical manifestations in people with *Blastocystis* from acute watery diarrhoea through mild chronic abdominal discomfort (abdominal pain, nausea, bloating, constipation, flatulence), to a total lack of symptoms (Dogruman-Al et al., 2010). The contribution of *Blastocystis* in irritable bowel syndrome (IBS) and irritation bowel disease (IBD) development is also ongoing debate

* Corresponding author at: Medical University of Gdańsk, Department of Tropical Medicine and Epidemiology, Powstania Styczniowego 9B, 81-519 Gdynia, Poland.
E-mail address: mrudz@gumed.edu.pl (M. Rudzińska).

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(Yakoob et al., 2010; Fouad et al., 2011; Poirier et al., 2012). Extra-intestinal symptoms such as a headache, chronic fatigue, itching and skin rash, and even depression have been described as well (Tan, 2008). The extent and severity of symptoms may be the result of the pathogenicity of the strain and the immunological status of the infected person (Clark et al., 2013; Alinaghizade et al., 2017; Seyer et al., 2017). The opportunistic potential of *Blastocystis* cannot be ruled out because it is frequently found in immunocompromised patients (cancer patients or HIV positive individuals) (Tan et al., 2009; Poirier et al., 2011).

Molecular epidemiology studies of *Blastocystis* infections have been conducted in many regions of the world, showing geographic variation in the prevalence and distribution of STs as well as conflicting data on the association of particular STs with clinical symptoms (Clark et al., 2013). In Poland, knowledge about the distribution of *Blastocystis* STs is very scarce. To date, there are only a few reports describing small groups of *Blastocystis*-positive subjects (Wesolowska et al., 2016; Lepczyńska et al., 2016; Sałamatin et al., 2016; Kaczmarek et al., 2017).

In this study, we aimed to investigate the distribution of *Blastocystis* STs among Polish citizens in relation to symptoms of the gastrointestinal tract (GIT) and history of travels to the warm zone. We hope it will help to enrich knowledge about the geographical distribution, transmission and the potential correlation between *Blastocystis* STs and disease.

2. Material and methods

A total of 122 *Blastocystis* positive stool samples collected from patients of the Tropical Medicine and Epidemiology Department (TM&ED) of the Medical University in Gdańsk in 2012–2013 were analysed. The study group consisted of people after returning from the tropics ($n = 92$), and people who had never left Poland ($n = 30$) independently of clinical symptoms. All samples were obtained during routine microscopic stool examination for ova and cysts (saline and iodine wet mounts, Kato-Katz and formol-ethyl-acetate-concentration methods) performed three times at a one – two-day interval. In the microscopic assessment of the intensity of *Blastocystis* invasion (using a 40× objective), four levels of parasite load (according to system developed in the TM&ED) were used: I - very low: single cells in a whole preparation; II - low: single cells in almost every field of vision; III - medium: 5–10 cells in every field of vision; IV – high: > 10 cells in every field of vision. The parasite load varied in individual patients in subsequent stool examinations. To assess accurately the potential association between the intensity of invasion and the occurrence of clinical symptoms, the sample with the highest level of parasite for each patient were used for examination. A standardized questionnaire was designed to collect information about each participating subject including sex, age, history of travels and presence of digestive disorders.

Stool samples were cultured in Jones' medium (Jones, 1946) supplemented with 10% cattle serum (Sigma-Aldrich Biomed, Poland) at 37 °C. After 48 h, each culture was screened for the presence of *Blastocystis* cells with a light microscope using a 40× objective and then centrifuged at 500 ×*g* for 5 min. The pellet was washed two times with 0.9% saline. The supernatant was discarded, and the pellet was frozen at −20 °C. DNA was extracted from the pellet using the Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instruction. Subtyping was performed by polymerase chain reaction (PCR) with seven pairs of subtype-specific sequence-tagged-site (STS) primers (SB83, SB155, SB227, SB332, SB340, SB336, and SB337) described by Yoshikawa et al. (2004). The nomenclature of the subtypes used in this report is according to the consensus terminology for *Blastocystis* STs described by Stensvold et al. (2007) (Table 1). DNA amplification was carried out in 50 µl reaction mixtures consisting of 5 µl of PCR buffer (10× concentration), 5 µl of dNTP mixture (the concentration of each dNTP was 2.5 mM), 1 µl of each primer (a concentration 10 µM each), 1 U of RUN polymerase (A&A Biotechnology, Poland), 2 µl of genomic DNA, and 35 µl of distilled water. The PCR conditions were all the same for all primer sets and consisted of the following steps: initial activation for 3 min at 94 °C; 30 cycles of denaturation for 30 s at 94 °C, annealing 30 s at 59 °C, elongation 1 min at 72 °C, followed by a final elongation step for 5 min at 72 °C and a hold step at 4 °C in a thermal cycler (Mastercycler, Eppendorf, Germany). The PCR products and molecular marker (100 base-pair ladder, Fermentas, USA) were electrophoresed on a 1.5% agarose gel (Sigma, St. Louis, Missouri) at 150 V for 40 min with Tris-borate-EDTA buffer. Bands were visualized by ethidium bromide using an ultraviolet gel documentation system GelDoc-It.

Statistical analysis and mathematical modelling were performed using the R programme (R Core Team, 2015). The procedures and functions for creation frequency and contingency tables, a graphical representation of the results, and independence tests have been taken from the "vcdExtra" package of this software. In all analyses, a significance level < 0.05 was regarded as statistically significant. The Fisher's Exact Test for count data for "n" given in Tables 2–6 was used to check the independence of the considered characteristics. The Wilcoxon rank sum test (for two groups) and Kruskal-Wallis test (for more groups) were used to examine the intensity of invasion with respect to disorders, traveling and non-leaving Poland, and *Blastocystis* STs, the length of stay abroad as well as the age of individuals.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki, and its later amendments or comparable

Table 1
Primers used in our study.[a]

Subtypes names	STS primer sets	Sequences of forward (F) and reverse (R) primers (5′ to 3′)
ST1	SB83	F - GAAGGACTCTCTGACGATGA R - GTCCAAATGAAAGGCAGC
ST2	SB340	F - TGTTCTTGTGTCTTCTCAGCTC R - TTCTTTCACACTCCCGTCAT
ST3	SB 227	F - TAGGATTTGGTGTTTGGAGA R - TTAGAAGTGAAGGAGATGGAAG
ST4	SB 337	F - GTCTTTCCCTGTCTATTCTGCA R - AATTCGGTCTGCTTCTTCTG
ST5	SB336	F - GTGGGTAGAGGAAGGAAAACA R - AGAACAAGTCGATGAAGTGAGAT
ST6	SB 332	F - GCATCCAGACTACTATCAACATT R - CCATTTTCAGACAACCACTTA
ST7	SB 155	F - ATCAGCCTACAATCTCCTC R - ATCGCCACTTCTCCAAT

[a] According to consensus nomenclature by Stensvold et al. (2007) which is different from that in the original primer description by Yoshikawa et al. (2004).

Table 2
The occurrence of *Blastocystis* STs depending on sex, and travels outside the borders of Poland.

		Blastocystis STs							p-value[a]
		ST1	ST2	ST3	ST6	ST7	ST1/ST3	Total	
Female	n	3	5	20	2	2	2	34	0.134
	%r	8.8	14.7	58.8	5.9	5.9	5.9	100	
Male	n	13	19	52	2	2	0	88	
	%r	14.8	21.6	59.1	2.3	2.3	0	100	
Not-leaving Poland	n	0	3	21	3	3	0	30	0.0008
	%r	0	10	70	10	10	0	100	
Travelers	n	16	21	51	1	1	2	92	
	%r	17.4	22.8	55.4	1.1	1.1	2.2	100	
Total	n	16	24	72	4	4	2	122	
	%r	13.1	19.7	59	3.3	3.3	1.6	100	

n - number of elements in subgroups, %r - percentage of the number relative to the sum of counts for the given row.

[a] Fisher's exact test.

Table 3
The occurrence of *Blastocystis* STs in travelers depending on the length of stay abroad.

			Blastocystis STs							p-value[a]
			ST1	ST2	ST3	ST6	ST7	ST1/ST3	Total	
Stay abroad	Long	n	13	14	39	0	1	2	69	0.4732
		%r	18.8	20.3	56.5	0	1.4	2.9	100	
Travelers	Short	n	3	7	12	1	0	0	23	
		%r	13	30.4	52.2	4.3	0	0	100	
	Total	n	16	21	51	1	1	2	92	
		%r	17.4	22.8	55.4	1.1	1.1	2.2	100	

n - number of elements in subgroups, %r - percentage of the number relative to the sum of counts for the given row.
[a] Fisher's exact test.

Table 4
Blastocystis STs detected in individuals after returning from different continents.

Continent		*Blastocystis* STs							p-value[a]
		ST1	ST2	ST3	ST6	ST7	ST1/ST3	Total	
Asia[b]	n	9	14	31	0	1	1	56	0.8642
	%r	16.1	25	55.4	0	1.8	1.8	100	
Africa	n	6	4	14	1	0	1	26	
	%r	23.1	15.4	53.8	3.8	0	3.8	100	
South America	n	0	2	3	0	0	0	5	
	%r	0	40	60	0	0	0	100	
All above continents	n	1	1	3	0	0	0	5	
	%r	20	20	60	0	0	0	100	
Total	n	16	21	51	1	1	2	92	
	%r	17.4	22.8	55.4	1.1	1.1	2.2	100	

n - number of elements in subgroups, %r - percentage of the number relative to the sum of counts for the given row.
[a] Fisher's exact test.
[b] Stays in south-eastern Asia, mainly India.

Table 5
The occurrence of *Blastocystis* STs depending on presence or absence of disorders.

		Blastocystis STs						p-value[a]
		ST1	ST2	ST3	ST6	ST7	Total	
Symptomatic[b,c]	n	8	9	23	1	2	43	0.6549
	%r	18.6	20.9	53.5	2.3	4.7	100	
Asymptomatic[c]	n	8	11	46	3	2	70	
	%r	11.4	15.7	65.7	4.3	2.9	100	
Total	n	16	20	69	4	4	113	
	%r	14.2	17.7	61.1	3.5	3.5	100	

n - number of elements in subgroups, %r - percentage of the number relative to the sum of counts for the given row.
[a] Fisher's exact test.
[b] Not included seven individuals with symptoms of GIT who apart from *Blastocystis* had other parasitic protozoa (including one with *E. histolytica* sensu *lato*).
[c] Not included one individual with mixed infection.

Table 6
The occurrence of *Blastocystis* STs depending on the age.

Age groups		*Blastocystis* STs							p-value[a]
		ST1	ST2	ST3	ST6	ST7	ST1/ST3	Total	
< 20–30 >	n	9	7	19	0	1	1	37	0.5671
	%r	24.3	18.9	51.4	0	2.7	2.7	100	
< 31–40 >	n	2	7	20	1	2	0	32	
	%r	6.25	21.88	62.5	3.13	6.25	0	100	
< 41–50 >	n	3	3	14	0	0	0	20	
	%r	15	15	70	0	0	0	100	
> 51	n	2	7	19	3	1	1	33	
	%r	6.1	21.2	57.6	9.1	3	3	100	
Total	n	16	24	72	4	4	2	122	
	%r	13.1	19.7	59	3.3	3.3	1.6	100	

n - number of elements in subgroups, %r - percentage of the number relative to the sum of counts for the given row.
[a] Fisher's exact test.

ethical standards. This study had the approval of the Independent Bioethics Commission for research at the Medical University of Gdansk. All participants provided written consent.

3. Results

The study group ($n = 122$) included 34 female and 88 male subjects; 30 had never left Poland, and 92 had had an experience of traveling to various zones with hot climates, with 23 and 69 having < 1 month' (tourists) and > 5 months' (occupational activity) stay time (respectively). 71 were asymptomatic, and 51 complained of a variety of symptoms mainly of the GIT. Gastrointestinal protozoa were detected microscopically in 24 individuals: 15 of them had pathogenic species (*Dientamoeba fragilis, Giardia intestinalis*), while eight had non-pathogenic ones (*Entamoeba coli*, *Endolimax nana*). *Entamoeba histolytica /dispar /moshkovski* (microscopically indistinguishable) was found in one person. Seven individuals with symptoms of the GIT who had parasitic protozoa (including one with suspicion of pathogenic *E. histolytica*) were not included in the clinical assessment. Similarly, to avoid possible disturbance of mixed *Blastocystis* STs, two patients co-infected with ST1 and ST3 were excluded from the clinical assessment. Thus, the clinical evaluation was based on 113 subjects (70 symptomatic, 43 asymptomatic). The patients were divided into age groups: 20–30, 31–40, 41–50 and over 50 years old. The mean age of participants was 40 years (the age range was 20 to 80 years).

Screening of the 122 *Blastocystis*-positive stool cultures with the seven pairs of STS primers revealed five STs: ST1, ST2, ST3, ST6, ST7. ST3 was the most abundant ($n = 72$, 59.0%) followed by ST2 ($n = 24$, 19.7%) and ST1 ($n = 16$, 13.1%). ST6 and ST7 were identified in 4 patients (3.3%) each. Two patients revealed mixed infections with ST1/ST3 (1.6%) (Fig. 1). The occurrence of particular STs depending on sex and travel outside the borders of Poland was summarized in Table 2. A statistically significant difference was found only in the occurrence of ST1 between the group of travelers and people not leaving Poland ($p = .0008$). No association was found between the length of stay abroad and *Blastocystis* STs detected in travelers (p-value = .4732) (Table 3). Table 4 shows the *Blastocystis* STs detected in individuals after returning from different continents. There was no association (p-value = .6549) between *Blastocystis* STs and the presence or absence of disorders (Table 5).

No statistically significant differences were found between the intensity of *Blastocystis* invasion and symptoms (p-value = .3205), travels (p-value = .4121), the length of stay abroad (p-value = 1), *Blastocystis* STs (p-value = .1384), and the age of participants (p-value = .6136) (Supplementary Figs. 1–5). Similarly, no significant differences were

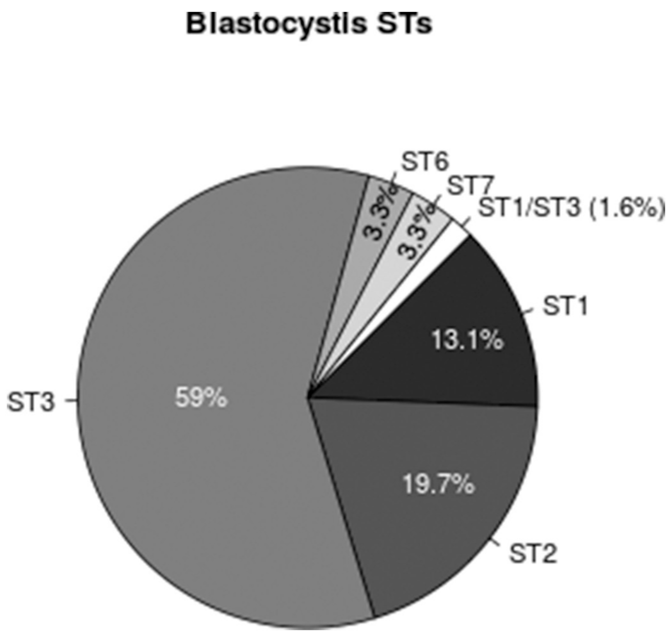

Fig. 1. *Blastocystis* STs distribution in the surveyed group.

found between the age of the patients and *Blastocystis* STs (p-value = .5671) (Table 6).

4. Discussion

Numerous reports from different world regions show that *Blastocystis* is one of the most frequently reported parasite in human intestines (Stensvold and Clark, 2016). This is in line with the observations being conducted in our department since 1992, which have shown that *Blastocystis* is the most commonly observed parasite in the stool samples of our patients. The number of patients with *Blastocystis* has increased 14-fold during that time – from 1.34% to 18.8% – while the incidence of other reported gastrointestinal parasites such as *Entamoeba histolytica/dispar*, *Giardia intestinalis*, *Dientamoeba fragilis*, *Ascaris lumbricoides*, *Enterobius vermicularis*, *Trichuris trichiura* and *Taenia* sp. has shown a marked downward trend (Kowalewska et al., 2013). Up to now reports on the distribution of *Blastocystis* STs in Poland have been scarce. ST1, ST2, ST3, ST4, ST6, ST7 were reported in small groups of subjects (from 6 to 31) (Lepczyńska et al., 2016; Sałamatin et al., 2016; Kaczmarek et al., 2017). ST3 was the most numerous in all these reports, followed by ST1, and rarely ST6 and ST7. Wesołowska et al. (2016) identified only ST3.

4.1. STs distribution in travelers and non-travelers

In our study, five *Blastocystis* STs were identified: ST3, followed by ST2 and ST1. The least numerous were ST6 and ST7 (Fig. 1) which is in line with the worldwide STs distribution (Alfellani et al., 2013). ST3 was predominant in both groups: travelers and those who never left Poland. This subtype is probably of human origin and its predominance in humans was explained by large-scale human-to-human transmission (Tan, 2008; Kaneda et al., 2001; Souppart et al., 2010). Our study has shown that ST2 in travelers was over twice as likely as in people never leaving Poland, but the difference was not statistically significant (Table 2). ST2 prevailed in individuals returning from South America and Asia, and the lowest number was found in those returning from Africa (Table 4). A literature review on the prevalence of ST2 shows that the distribution of this subtype in different continents is very diverse. Usually it occurs less often than ST3 and ST1, but in many reports it was not detected (Alfellani et al., 2013; Piubelli et al., 2019). On the other hand, in some countries of South America it was as frequent as ST3 (Ramírez et al., 2016). Therefore, the question of ST2 import requires further investigation. It is intriguing in our study that ST1 was only found in travelers (17.4%) after returning from Africa and Asia, suggesting a possibility of ST1 being imported to Poland from these continents. However, we cannot draw a definitive conclusion, for the participants were not examined before their stay in the tropics. Similarly, Bart et al. (2013) observed a more frequent occurrence of ST1 in patients of the Tropical Disease Department than other clinical departments of the Academic Medical Centre in Amsterdam. The report of Alfellani et al. (2013), who gathered data about subtype distribution all over the world, shows that ST1 generally is less frequent in Europe than in other continents, although in single reports from Germany, Greece and Denmark it was second in terms of frequency. However, the shortcoming of majority of reports from Europe is the lack of data on the travel history of *Blastocystis*-positive individuals, which does not allow to conclude on the geographical origin of ST1 in Europe. The only report containing data on the pre-and post-travel occurrence of *Blastocystis* shows that *Blastocystis* carriage in travelers is highly dynamic (van Hattem et al., 2018). In this study ST1 was the most frequently acquired subtype after travel to Africa but it also occurred in many patients before travel, however there is no information whether the travel was the first or subsequent of a given traveler. ST6 and ST7 are rarely met in humans, slightly more in Asia than Europe (Alfellani et al., 2013; Forsell et al., 2012). They are considered to be of bird origin (Stensvold and Clark, 2016; Alfellani et al., 2013; Ramírez et al., 2014; Cian et al., 2017) but they were also detected in *Bovidae*, less frequently in carnivores, and ST7 in non-human primates (Cian et al., 2017). Therefore, to explain whether human infections caused by ST6 and ST7 are the result of zoonotic transmission, more research is needed comparing ST6 and ST7 obtained from humans and animals. The lack of ST5 in the present study was not a surprise since ST5 has mostly been reported in livestock. In humans all over the world, ST5 is detected sporadically, mainly in Asia in people living near farms (Yan et al., 2007). The low transmission of ST5 from animals to humans in frequent and close contact with each other may suggest high host-specificity for ST5 and it needs further research. We were most surprised by the absence of ST4 in the surveyed group, since ST4 has been reported in all European countries where such studies have been conducted. In single reports from Denmark, France, and Spain, it was the most numerous while outside of Europe ST4 is rarely detected (Alfellani et al., 2013). In the abovementioned Polish studies on *Blastocystis* STs, ST4 was identified only in one of four reports in small percentage of samples (Lepczyńska et al., 2016).

To sum up, the STs distribution in our surveyed group was more like the STs from outside Europe than those from Europe, suggesting that *Blastocystis* infection was probably acquired during the stay out of Poland The STs distribution was not affected by the length of stay abroad (Table 3).

4.2. Blastocystis STs and clinical symptoms

To date, the pathogenicity of *Blastocystis* and blastocystosis related to STs still remains unclear. There are as many reports showing the connection between *Blastocystis* STs and the occurrence of disorders, as well as those denying these findings. In our study, there were no differences in STs distribution in patients with and without disorders. (Table 5). Similar results were found by Yoshikawa et al. (2004), Alinaghizade et al. (2017), Seyer et al. (2017), Souppart et al. (2009), Souppart et al. (2010), Ozyurt et al. (2008), Motazedian et al. (2008). On the other hand Kaneda et al. (2001), Yan et al. (2006) and Hussein et al. (2008) reported the occurrence of symptoms in individuals colonized by ST1, and Abdel-Hameed and Hassanin (2011), Hameed et al. (2011) and Mohamed et al. (2017) in those colonized by ST3. Kaneda et al. (2001) in turn suggested a relation of ST2 and ST4 with disorders of the GIT. The ST4 association with bowel disorders and diarrhoea was also noted by Domínguez-Márquez et al. (2009) and Stensvold et al. (2011). The latter believes ST4 as the only subtype associated with the occurrence of diarrhoea and IBS.

Seeking the causes of differential clinical symptoms or their absence in infected patients, the development of disorders depending on the parasite load has been suggested. Fathy (2011), Tan (2008) and EL-Marhoumy et al. (2015) noticed a positive correlation between the intensity of invasion and the clinical presentation, accepting the intensity of above 5 organisms per field of vision as a pathogenic criterion. On the other hand Poirier et al. (2011) Ozyurt et al. (2008), and Doyle et al. (1990) did not find such a correlation between them. Statistical analysis of our data did not show any significant differences between the parasite load and the presence of disorders, history of travels, the length of stay abroad or *Blastocystis* STs (Supplementary Figs. 1–4). The issue of the potential relationship between the above factors requires further research.

We did not find an age predisposition for the *Blastocystis* infection. The average age of our patients was 40 years (ranged 20–80 years) in both (symptomatic and asymptomatic) groups. The same conclusion was drawn by Scanlan et al. (2014) in analysing asymptomatic carriers, and by Yakoob et al. (2010) in analysing patients with *Blastocystis* and IBS. In contrast, Doyle et al. (1990) observed the highest infection rate in patients who were 33–34 years old. Children seem to be less infected; however, the highest presence of *Blastocystis* ever observed worldwide (100%) was in a group of 93 children in the Senegal River Basin (El Safadi et al., 2014). In our study the intensity of invasion and infection with particular STs did not depend on the age of patients (Supplementary Fig. 5).

Some researchers tried to connect the occurrence of symptoms in *Blastocystis* infected individuals with the morphological form of the parasite. Tan (2008), Tan and Suresh (2006), Katsarou-Katsari et al. (2008) and Vassalos et al. (2010) noted that amoeboid form occurred only in people with symptoms, thus this form may be responsible for the development of disorders. In the microscopic examination of our patients' samples both with and without symptoms, we observed only vacuolar forms. Therefore, we cannot support the above hypothesis.

5. Conclusion

We established the distribution of *Blastocystis* STs among Poles traveling to different continents and never leaving Poland. Five *Blastocystis* STs were identified, with a predominance of ST3 in both groups. ST1 was identified only in the group of travelers to hot climate zones, and ST2 occurred twice as often in this group than in non-travelers. Our results showed no association between particular STs and clinical symptoms. The crucial value of our research is the observation that certain Blastocystis STs (here ST2, and especially ST1) might have been acquired during stays outside of Europe which sheds more light on the issue of Blastocystis infection import. These data may be the first step to decipher and explain the origin of Blastocystis STs in Europe, and show that transmission of the parasite requires further research especially in the context of the increasing number of people traveling to hot climate zones. Accurate data on the travel history as well as pre- and post-travel Blastocystis carriage of the persons surveyed are necessary for clarification of which STs detected in Europe are indigenous and which ones could be imported due to travel or migration.

Declarations of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103926.

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