Infection, Genetics and Evolution 37 (2016) 158–162

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Prevalence and subtype distribution of *Blastocystis* in healthy individuals in Sharjah, United Arab Emirates

Raed AbuOdeh [a], Sinda Ezzedine [a], Amidou Samie [b], Christen Rune Stensvold [c], Ali ElBakri [a,*]

[a] Medical Laboratory Sciences Department, College of Health Sciences, University of Sharjah, Sharjah, United Arab Emirates
[b] Molecular Parasitology and Opportunistic Infections Program, Department of Microbiology, University of Venda, Thohoyandou, South Africa
[c] Laboratory of Parasitology, Department of Microbiology and Infection Control, Statens Serum Institut, Artillerivej 5, DK 2300 Copenhagen S, Denmark

ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form 15 November 2015
Accepted 20 November 2015
Available online 27 November 2015

Keywords:
Blastocystis
PCR
Subtyping
Molecular epidemiology

ABSTRACT

Blastocystis is estimated to be one of the most common parasites of the intestinal tract of humans, comprising multiple subtypes (ST). Meanwhile, the distribution of *Blastocystis* ST in many communities and countries remains unknown. In the present work, we aimed to identify the prevalence of *Blastocystis* and the ST distribution in human stool samples collected from healthy expatriates from different geographical regions and residing in Sharjah, United Arabian Emirates (UAE). A total of 133 samples were screened and subtyped using partial small subunit ribosomal RNA gene sequencing. Fifty-nine (44.4%) samples were identified as positive. Among these, 39 were successfully sequenced and subtyped. The ST distribution was as follows: ST3, 58.9% (23/39); ST1, 28.2% (11/39); and ST2, 7.6% (3/39). No correlation between geographic origin and infection ($\chi^2 = 11.006$; $P = 0.528$) nor gender and infection ($\chi^2 = 1.264$; $P = 0.261$) was observed. The data were compared with those available for other Middle Eastern and North African neighboring countries. This study is the first to provide data concerning the prevalence of *Blastocystis* and the frequency of various STs in the UAE, confirming the absence of ST4 and the commonness of ST1, ST2, and ST3 in this geographical region.

1. Introduction

Blastocystis is an intestinal parasite often observed in human fecal samples. It has a worldwide distribution with a prevalence of up to 100% in developing countries (El Safadi et al., 2014) and up to at least 56% in developed countries (Scanlan et al., 2014). A higher risk of infection has been identified in food and animal handlers, which may suggest transmission of the organism between humans and animals (Parkar et al., 2010). Stensvold et al. (2007) classified distinct ribosomal lineages of *Blastocystis* into subtypes (STs) – arguably separate species – based on small subunit ribosomal RNA gene (SSU rDNA) analysis, representing genetically diverse *Blastocystis* species isolated from humans, other mammals and birds; currently, a total of 17 STs are known (Alfellani et al., 2013a). The majority of human *Blastocystis* carriage is attributable to ST3, but infection with ST1, ST2 and ST4 are also common (Souppart et al., 2009; Alfellani et al., 2013b). Moreover, the distribution of these four predominant STs may vary depending on the geographic areas studied (Alfellani et al., 2013b). While ST5–ST9 have been isolated only sporadically from humans (Li et al., 2007; Meloni et al., 2011; Alfellani et al., 2013a), ST10–ST17 have not been reported in humans to date (Alfellani et al., 2013b). The pathogenic potential of *Blastocystis* is still controversial, and many carriers probably remain asymptomatic or exhibit symptoms ranging from slight intestinal discomfort to disease manifestations such as acute or chronic diarrhea, abdominal pain, flatulence, vomiting, irritable bowel syndrome and inflammatory bowel disease (Jimenez-Gonzalez et al., 2012; Tan et al., 2010; Poirier et al., 2012). It has long been suggested that the pathogenesis of *Blastocystis* sp. may be dependent upon ST, but recent epidemiological data remain contradictory (El Safadi et al., 2013; Clark et al., 2013). Consequently, information on the prevalence of the parasite and the distribution of STs is beginning to emerge in still poorly studied geographic areas.

Few studies have reported the presence of *Blastocystis* from healthy asymptomatic individuals, adding to the uncertainty regarding the pathogenic nature of this organism (Pandey et al., 2015; Scanlan and Stensvold, 2013; Markell and Udkow, 1990; Zierdt, 1991). Furthermore, data on the different STs in asymptomatic individuals are needed to increase our understanding of STs possibly linked to symptomatic infections.

Studies on *Blastocystis* in the Middle East and the Arabian Gulf countries are limited, and most available data were derived from examination of direct fecal smears (Abu-Madi et al., 2010). To our knowledge, no study investigating the prevalence and ST distribution of *Blastocystis* in the United Arab Emirates (UAE) has ever been performed. Therefore, the aim of the present study was to investigate *Blastocystis* prevalence and ST distribution in the UAE by performing the first survey on this

* Corresponding author at: Medical Laboratory Sciences Department, College of Health Sciences, University of Sharjah, PO Box 27272, Sharjah, United Arab Emirates.
E-mail address: aelbakri@sharjah.ac.ae (A. ElBakri).

http://dx.doi.org/10.1016/j.meegid.2015.11.021
1567-1348/

parasite using fecal samples collected from healthy expatriates from different localities residing in Sharjah, UAE.

2. Materials and methods

2.1. Stool samples

A total of 133 stool samples from asymptomatic individuals representing 13 countries were collected as part of a mandatory pre-employment testing for intestinal parasites at the Sharjah Municipality Public Health Clinic (SMPHC) between June 2009 and January 2012. The group included expatriate workers of both sexes with a mean age of 32.9 years (±9.3). Approximately 1–5 g of stool was collected in sterile leak-proof plastic containers with no preservative and transported without delay to the University of Sharjah (Sharjah, UAE) where aliquots of fresh, unpreserved stool samples were stored at −20 °C until used for DNA extraction and PCR. Where possible, epidemiological data (nationality, age, and gender) were obtained from the SMPHC. For the purpose of analysis, samples were grouped into four geographical regions of origin as follows (constituent countries and number of subjects are given in parenthesis): Western Asia (Afghanistan, $N = 10$; India, $N = 50$; Bangladesh, $N = 26$; Pakistan, $N = 14$; Nepal, $N = 5$; Sri Lanka, $N = 3$), Eastern Asia (Philippines, $N = 7$; Indonesia, $N = 3$), Middle East (Egypt, $N = 5$; Jordan, $N = 1$; Sudan, $N = 1$), and Africa (Ethiopia, $N = 7$; Tanzania, $N = 1$).

2.2. DNA extraction and PCR amplification of the Blastocystis SSU rRNA gene

DNA was extracted from all stool samples using the QIAamp stool DNA Mini Kit (Qiagen GmbH, Hilden, Germany) as per manufacturer's recommendations and stored at −20 °C until analyzed. PCR amplification conditions of the 5′-end 600-bp region of the *Blastocystis* SSU rRNA gene using the *Blastocystis*-specific reverse primer BhRDr (5′-GAGCTTTTTAACTGCAACAACG-3′) and the broad-specificity eukaryote-specific forward primer RD5 (5′-ATCTGGTTGATCCTGCCAGT-3′) were applied as described by (Scicluna et al., 2006; Stensvold, 2013). Briefly, the amplification conditions consisted of 30 cycles of 1 min each at 94 °C, 59 °C, and 72 °C, with an additional 2-min final extension. PCR products were separated on 1.2% agarose gels stained with ethidium bromide. Positive and negative (DNA replaced by water) controls were included with each batch of samples analyzed.

2.3. DNA sequencing, phylogenetic analysis, and molecular subtyping of Blastocystis

PCR products from 39 representative isolates were purified using the Qiaquick gel extraction kit (Qiagen) according to the manufacturer's instructions and sent for sequencing in a sequencing facility (Inqaba Biotech) in Pretoria, South Africa. PCR products were sequenced on both strands, and the resulting sequences were assembled online using http://www.prabi.fr/, and the assembled contigs were aligned using the ClustalW2 multiple sequence alignment tool available at http://www.ebi.ac.uk/Tools/msa/clustalw2/. For phylogenetic analysis, SSU-rRNA sequences from 37 isolates were analyzed together with an outgroup (*Proteromonas lacertae*, Genbank accession no. U37108) and the evolutionary relationship between taxa was constructed using MEGA5 software. A neighbor-joining tree was generated on the basis of the evolutionary distances calculated by the Tamura-Nei model using the MEGA5 program (Tamura and Nei, 1993; Tamura et al., 2011). Bootstrap values were calculated by analyzing 1000 replicates. Sequences were compared with *Blastocystis* SSU rDNA sequences available from the National Centre for Biotechnology Information (NCBI) by BLAST queries. Additionally, *Blastocystis* STs were identified by determining the exact match or closest similarity against all known *Blastocystis* sp. STs using www.pubmlst.org/blastocystis.

2.4. Statistical analysis

Data were analyzed statistically (Chi-square test) using IBM SPSS Statistics for Windows v.21 (IBM Corp., Armonk, NY).

3. Results

3.1. Identification of Blastocystis

Of the 133 samples tested, 59/133 (44.4%) were identified as positive for *Blastocystis* SSU rDNA, with 74/133 (55.6%) being negative. Hence, 59 isolates generated the expected fragment size (600 bp) with a high yield. All representative isolates were confirmed by sequence analysis and corresponded to the already described SSU-rRNA isolates in GenBank. Moreover, no correlation between geographic origin and infection ($\chi^2 = 11.006$; $P = 0.528$) nor gender and infection ($\chi^2 = 1.264$; $P = 0.261$) were noted.

The expected SSU rDNA target was amplified and sequenced for all representative isolates. All 39 sequenced isolates corresponded to already deposited SSU rDNA sequences in GenBank. Fig. 1 shows the phylogenetic tree obtained with maximum likelihood analysis of the aligned SSU rDNA sequences. The nucleotide sequence PLU37108 *Proteromonas lacertae* was used as an outgroup. When the phylogenetic reconstruction was completed, an accurate discrimination of *Blastocystis* sp. STs compatible with that obtained by BLAST queries at www.pubmlst.org/blastocystis was found (Table 1). Among a total of 39 subtyped isolates, ST3 was most abundant (58.9% [23/39]), followed by ST1 (28.2% [11/39]) and ST2 (7.6% [3/39]). While 37 isolates produced single subtypes, it was not possible to clearly ascertain the subtypes of two of the isolates. It is probable that one of them had a mixed subtype infection and the other one possibly was an ST3 but with mixed alleles (perhaps containing two different ST3 strains mixed together. No other subtypes were found in the representative isolates.

4. Discussion

In the present study, we have shown for the first time that the infection rate with *Blastocystis* sp. as assessed by PCR and sequencing is 44.4% among asymptomatic expats in Sharjah, UAE. Although the PCR used in this study is not diagnostic, all of the 39 randomly chosen representative PCR products were confirmed to represent *Blastocystis* by sequencing, and so we have reason to believe that the remaining 20 samples were also 'true positives'.

Comparison of our findings with those from other Middle Eastern and North African countries showed that the prevalence reported in UAE was higher compared with that reported in Jordan (25%), Libya (30%), and Lebanon (19%) (Nimri, 1993; Al-Fellani et al., 2007; El Safadi et al., 2013). These differences were probably due to differences in methods used. Unlike the present study, less sensitive non-DNA based methods were used in the other studies.

To date, few studies have been performed with regard to the epidemiology of *Blastocystis* in the Arabian Gulf region. In Qatar, Abu-Madi et al. (2010) reported a prevalence of 2.9% infection rate with *Blastocystis* sp. in both new immigrants and residents combined. Moreover, a study investigating the prevalence of *Blastocystis* in food handlers in Dammam, Eastern Province of Saudi Arabia reported a prevalence of 8.5% (Khan and Alkhalife, 2005). Similar to our study group, the population in those studies were a heterogeneous asymptomatic expatriate populations arriving from India, Pakistan, Philippines, Sri Lanka, and other Arab countries and mainly employed and/or seeking employment in the UAE as food handlers, housemaids, and baby sitters.

This study showed no significant association between age, gender, or nationality of the participants and *Blastocystis* infection. This is in agreement with results from previous reports (Abdulsalam et al., 2012). However, Li et al. (2007) demonstrated that individuals aged 60 years