Parasitology International 61 (2012) 719–722

Contents lists available at SciVerse ScienceDirect

Parasitology International

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

Short communication

Mixed human intra- and inter-subtype infections with the parasite *Blastocystis* sp.

Dionigia Meloni [a,b], Philippe Poirier [c], Cléa Mantini [a], Christophe Noël [d], Nausicaa Gantois [a], Ivan Wawrzyniak [c], Frédéric Delbac [c], Magali Chabé [a], Laurence Delhaes [a], Eduardo Dei-Cas [a], Pier Luigi Fiori [b], Hicham El Alaoui [c], Eric Viscogliosi [a,*]

[a] Pasteur Institute of Lille, Center for Infection and Immunity of Lille (CIIL), Inserm U1019, CNRS UMR 8204, University Lille Nord de France, EA4547, Lille Cedex, France
[b] Department of Biomedical Sciences, Division of Experimental and Clinical Microbiology, University of Sassari, Sassari, Italy
[c] Laboratoire Microorganismes: Génome et Environnement, CNRS UMR 6023, Clermont Université, Université Blaise Pascal, Aubière, France
[d] Geneius Laboratories Ltd, INEX Business Centre, Newcastle upon Tyne, United Kingdom

ARTICLE INFO

Article history:
Received 21 January 2012
Received in revised form 18 May 2012
Accepted 23 May 2012
Available online 29 May 2012

Keywords:
Blastocystis sp.
Intestinal parasite
Mixed infection
Molecular epidemiology
Subtype

ABSTRACT

Because of their limitations, current subtyping methods likely underestimate mixed human intra- and inter-subtype infections with *Blastocystis* sp. leading to erroneous data in the context of epidemiological studies. We confirmed this hypothesis by the identification of several isolates belonging to three subtypes in a patient considered at high risk of mixed infection through her lifestyle in rural area and long history of travelling.

Blastocystis sp. is an anaerobic enteric protist commonly occurring in humans and a wide range of animals [1]. It has a worldwide distribution and is often the most common intestinal parasite reported in human fecal samples [1,2]. The main mode of transmission of this parasite is the faecal-oral route through consumption of contaminated food and water [1]. Its pathogenic potential in humans remained debated because the infection can be asymptomatic. However, recent *in vitro* and *in vivo* data together with those of the analysis of its genome, allowed proposing a model for pathogenesis of this parasite [1,3]. Accumulating reports suggest an association between *Blastocystis* sp. and a variety of gastrointestinal disorders, including irritable bowel syndrome [4,5] and acute urticarial lesions associated with minor digestive symptoms [6]. This parasite has also increasingly been implicated for diarrheal illness in immunocompromised individuals including HIV and cancer patients [7]. Although morphologically indistinguishable, phylogenies inferred from SSU rRNA gene sequences revealed a considerable genetic divergence among *Blastocystis* sp. isolates from humans and animals and a total of 13 subtypes (STs) have so far been identified [8–10]. A majority of human infections with *Blastocystis* sp. are attributable to ST3 isolates [11,12] suggesting that ST3 may be the only ST of human origin [13]. All the others STs of supposed animal origin are zoonotic and almost all are able to infect humans in different frequencies. Therefore a higher risk of *Blastocystis* sp. infection has been shown in people living in rural area and/or with close animal contact [14].

Numerous epidemiological studies have reported the frequency of STs from symptomatic and asymptomatic individuals in several countries [1,11] in the aim i) to identify a possible link between ST and symptoms and/or gastrointestinal illnesses and ii) to track infection and contamination sources. For subtyping of *Blastocystis* sp. isolates, polymerase chain reaction (PCR), employing ST-specific primers, is a commonly used method. However, this approach allows only the amplification of 7/13 STs [15]. Isolates can also be characterized by amplification of informative domains of the SSU rRNA gene using *Blastocystis* sp.-specific primers followed by Restriction Fragment Length Polymorphism (RFLP) [16]. However, major limitations of this approach are the lack of standardization of the PCR conditions, choice of primers, and mutations at restriction sites. Another method consists of genus-specific amplification of a SSU rDNA gene domain followed by either direct sequencing of the PCR product or cloning and sequencing of a limited number (usually 2) of arbitrarily selected clones [11]. These subtyping methods show their own limitations but have in common to underestimate the prevalence of mixed infections with more than one *Blastocystis* sp. ST as well as with isolates deriving from the same ST in a particular individual. However, despite this

* Corresponding author at: Institut Pasteur of Lille, Center for Infection and Immunity of Lille (CIIL), Inserm U1019, CNRS UMR 8204, University Lille-Nord de France, Biology and Diversity of Emerging Eukaryotic Pathogens, EA4547, 1 rue du Professeur Calmette, BP 245, 59019 Lille cedex, France. Tel.: +33 3 20 87 79 61; fax: +33 3 20 87 78 88.
E-mail address: eric.viscogliosi@pasteur-lille.fr (E. Viscogliosi).

1383-5769/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2012.05.012

obvious underestimation, it has been shown in various studies (see [1,11] for reviews) that the prevalence of mixed infections with two different STs could exceed 10% of samples surveyed. Consequently these methodological drawbacks could seriously distort the results and understanding of epidemiological studies.

To test this hypothesis, we analyzed the genetic diversity of *Blastocystis* sp. isolates in a French patient, a 60-years old woman, considered at high potential risk of mixed infection with this parasite. Indeed, the patient showed several risk factors for contamination such as living in a rural area, eating fruits and vegetables from her garden or picked up in the country, and buying food in local markets. Moreover, she has a long history of travelling (Malta, Cuba, USA, Maghreb, Réunion Island) during the five last years and ate local food during her trips. As food-handler in Hospital, the patient without any gastrointestinal or skin symptoms, presented for a routine parasitic stool examination. *Blastocystis* sp. was identified through direct light microscopy (×400) of wet smears. DNA extraction from stool sample and PCR using *Blastocystis* sp.-specific primers and High-fidelity *Taq* DNA polymerase (to prevent PCR artifacts) were performed as previously described [11]. The 600 bp-amplified domain of the SSU rDNA coding region has been shown to provide sufficient information for accurate subtyping [17]. The PCR fragment was purified, cloned into the pCR 2.1-TOPO vector (Invitrogen, Groningen, The Netherlands), and amplified in TOP10 competent cells. Fifty positive clones (Cl1 to Cl50) and not only two as in previous studies [11] were arbitrarily selected and sequenced. The SSU rRNA gene sequences obtained in this study have been deposited in GenBank under accession numbers JN942527 to JN942576. These sequences were aligned with available sequences of *Blastocystis* sp. isolates representative of ST1 to ST10 using the BioEdit v7.0.1 package. ST11 to ST13 SSU rRNA sequences were not available for this particular domain [10]. Maximum likelihood phylogenetic analysis of sequences was performed with PhyML 3.0 [18] using the GTR (general time

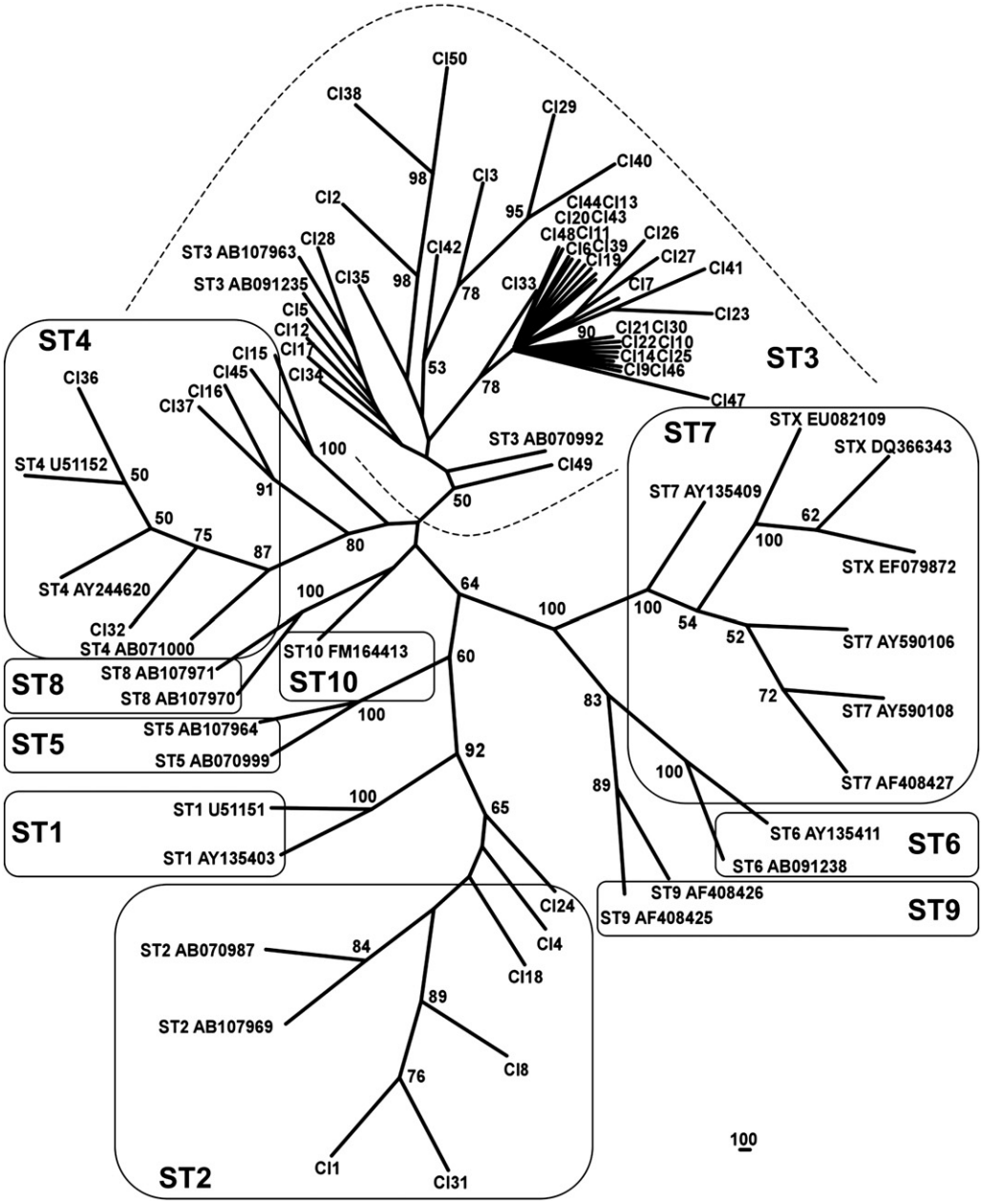

Fig. 1. Unrooted maximum likelihood tree of *Blastocystis* sp. isolates based on the 600 bp-domain sequences of the SSU rRNA gene. Numbers near the individual nodes indicate bootstrap values given as percentages. Values below 50% are not indicated. Clones sequenced in this study are called Cl1 to Cl50. Reference sequences from GenBank have the accession number preceded by the ST. The scale bar represents the average number of nucleotide replacements per site.