Prevalence and genetic diversity of *Blastocystis* in family units living in the United States

Pauline D. Scanlan, Rob Knight, Se Jin Song, Gail Ackermann, Paul D. Cotter

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Authors: Pauline D. Scanlan [1,2], Rob Knight[3], Se Jin Song[4], Gail Ackermann[3], and Paul D. Cotter [1,2].

Author affiliations:

[1]Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland

[2]APC Microbiome Institute, Biosciences Institute, University College Cork, Cork, Ireland

[3]Department of Pediatrics and Computer Science & Engineering, University of California at San Diego, San Diego, CA, USA

[4]Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, CO, USA

Correspondence: Pauline Scanlan, Present Address: APC Microbiome Institute, Biosciences Institute, University College Cork, Cork, Ireland. Telephone: 00353 (21) 4901335, Email: paulinescanlan@yahoo.co.uk or p.scanlan@ucc.ie

Abstract

The human gut is host to a diversity of microorganisms including the single-celled microbial eukaryote *Blastocystis*. Although *Blastocystis* has a global distribution, there is dearth of information relating to its prevalence and diversity in many human populations. The mode of *Blastocystis* transmission to humans is also insufficiently characterised, however, it is speculated to vary between different populations. Here we investigated the incidence and genetic diversity of *Blastocystis* in a US population and also the possibility of *Blastocystis* human-human transmission between healthy individuals using family units (N = 50) living in Boulder, Colorado as our sample-set. Ten of the 139 (~7%) individuals in our dataset were positive for *Blastocystis*, nine of whom were adults and one individual belonging to the children/adolescents group. All positive cases were present in different family units. A number of different *Blastocystis* subtypes (species) were detected with no evidence of mixed infections. The prevalence of *Blastocystis* in this subset of the US population is comparatively low relative to other industrialised populations investigated to date; however, subtype diversity was largely consistent with that previously reported studies of European populations. The distribution of *Blastocystis* within family units indicates that human-human transmission is unlikely to have occurred within families that participated in this study. It is not unexpected that, given the world-wide variation in human living conditions and lifestyles between different populations, both the prevalence of *Blastocystis* and its mode of transmission to humans may vary considerably.

Keywords: *Blastocystis*, parasite, microbial eukaryote, microbial survey, prevalence, gut microbe, microbial diversity, human-human transmission

Highlights:

- First PCR-based survey of *Blastocystis* in a US population using *Blastocystis* specific primers
- Prevalence of *Blastocystis* in this subset of the US population is comparatively low relative to other industrialised populations investigated to date
- *Blastocystis* subtype diversity is largely consistent with that previously reported from studies of European populations
- The distribution of *Blastocystis* within family units indicates that human-human transmission is unlikely to have occurred within families studied here.

1. Introduction

Blastocystis is a single-celled microbial eukaryote that is commonly found in the intestinal tract of a diverse range of non-mammalian and mammalian hosts including humans (Alfellani et al., 2013; Alfellani et al., 2013a; Tan, 2008). As a consequence of the controversial and unresolved role of *Blastocystis* in human intestinal disease and the increased awareness of the importance of the gut microbiome (and specific components of the gut microbiome) in human health and disease, research into *Blastocystis* has increased greatly in recent years (Andersen and Stensvold, 2015; Guinane and Cotter, 2013; Roberts et al., 2014; Scanlan, 2012; Scanlan and Stensvold, 2013; Sekirov et al., 2010). Although the application of sensitive molecular PCR based techniques has faciltated a better appreciation of the prevalence of *Blastocystis* in human populations, there remains a lack of reliable epidemiological data on the prevalence and genetic diversity of *Blastocystis* from many regions of the world (Alfellani et al., 2013) including the United States. Moreover, a number of questions relating to the basic ecology and epidemiology of *Blastocystis* including the mode of *Blastocystis* transmission to humans remain unanswered (Clark et al., 2013).

It is estimated that more than one billion people worldwide are colonised by *Blastocystis* (Andersen and Stensvold, 2016). However, there is considerable variation in prevalence rates between different populations; for example, recent reports have given positive prevalence rates of anywhere between ~24-100% for different populations living in Europe, the Middle East, India and Africa (AbuOdeh et al., 2016; Alfellani et al., 2013; Bart et al., 2013; El Safadi et al., 2014; Krogsgaard et al., 2015; Pandey et al., 2015; Scanlan and Stensvold, 2013; Scanlan et al., 2014). With respect to *Blastocystis* transmission to humans, three distinct modes of transmission have been proposed (Tan,

2008). Firstly, there is some evidence of zoonotic transmission; however, this is most likely to occur only in certain circumstances and for specific subtypes (STs), *e.g.* close contact with animals that are host to subtypes that can also colonise humans (Alfellani et al., 2013a; Parkar et al., 2010; Wang et al., 2014). Secondly, environmental sources such as drinking water may also be a potential source of *Blastocystis* (Leelayoova et al., 2008; Taamasri et al., 2000). Poor sanitation and unsuitable water treatment resulting in the consumption of contaminated water are likely to be contributory in this regard (Taamasri et al., 2000; Tan et al., 2010). Finally, direct human-to-human transmission through contact with infected individuals (directly or indirectly *via* contaminated food and water) has also been postulated (Anuar et al., 2013; Yoshikawa et al., 2000).

In this study, we analysed the prevalence and genetic diversity of *Blastocystis* in a subset of the US population and also investigated the possibility of human-to-human transmission of *Blastocystis* between healthy individuals using families as our sample-set. We deliberately chose families from a limited geographical area as we anticipated that, if human-human transmission of *Blastocystis* is possible, more of the same *Blastocystis* STs (alleles) would be shared within, rather than between, family groups. The use of families also facilitates investigation of specific patterns of transmission including vertical and/or horizontal transmission, for example, between mothers and children and between partners and/or children. Moreover, in addition to close human-to-human contact, family groups also possess several attributes that further the analysis of human-to-human transmission including shared environments and degree of genetic relatedness. Our sample-set also included dogs which also allowed for the investigation of dogs as carriers of *Blastocystis* and potential sources of transmission to humans.

2. Material and Methods

2.1 Overview of study and participants

The aim of our study was to survey the prevalence and genetic diversity of *Blastocystis* in a subset of the (healthy) US population and to investigate the possible human-human transmission of *Blastocystis* within families. Our samples were taken from a previous study that comprised 50 family units (FUs), (adults, *N* = 101; mean age, 34.8 years; children/adolescents, *N* = 38; mean age, 4.1 years) (Song et al., 2013), see Table 1. This dataset also included dogs, which we investigated as a possible source of zoonosis (*N* = 36). The FUs comprised 17 spousal units with children and no dogs, 9 spousal units with children and dogs, 12 spousal units with one or more dogs and no children, and 12 spousal units with neither children nor dogs (see Supplementary Table 1 for full details of all participants).

2.2 *Blastocystis* PCR and sequence analysis

Genomic DNA was extracted from faecal samples as outlined (Song et al., 2013). We used the primer set RD5 and BhRDr to amplify and sequence ~600bp of the SSU rRNA gene in order to survey the prevalence and diversity of *Blastocystis* STs in our samples according to standard protocol (Scanlan et al., 2014; Scicluna et al., 2006). This PCR reaction has a lower threshold of detection limit at *Blastocystis* cell densities of 10^3/cells per gram of faeces and at DNA concentrations of < 1ng/μL per PCR reaction (Scanlan et al., 2014). All sequence data was submitted to the online site http://pubmlst.org/Blastocystis/ to assign *Blastocystis* subtype and allele ID. Sequences

were then aligned and analysed in MEGA4 (5). We assessed the incidence of mixed infections using a newly developed ST-specific primer set as described (Scanlan et al., 2015).

3. Results

We detected *Blastocystis* in 10 of the 139 (~7%) individuals in our dataset, see Supplementary Table 1. We found no evidence of mixed *Blastocystis* infections using ST-specific primers. The cohort of individuals positive for *Blastocystis* could be further subdivided into two groups; adults and children/adolescents, with nine and one individual(s), respectively, positive for *Blastocystis*. Of the adults, eight were male and one female and the positive child/adolescent sample was a two year old female, see Supplementary Table 1. Each of the 10 individuals' positive for *Blastocystis* was a member of a different FU (i.e. each of the positive samples were randomly distributed across different FUs in the dataset). We detected a number of different *Blastocystis* alleles in our sample-set; ST1 (20%; allele 4), ST2 (30%; alleles 9 and 12) and ST3 (50%; alleles 34 and 36). All of the dog samples were *Blastocystis* negative.

4. Discussion

A renewed research interest in the microbial eukaryote *Blastocystis* has been largely driven by a greater appreciation of its genetic and phenotypic diversity, ubiquity in human and animal populations, and its potential role in both human health and disease (Andersen and Stensvold, 2015; Tan et al., 2010). Currently, there is little information on *Blastocystis* prevalence and diversity in US populations derived from sensitive molecular methodologies. Although this is a relatively small sample-set and the study particpants comprised both related and unrelated individuals (and therefore are not a randomly sampled population), our data indicates that the prevalence rate of *Blastocystis* in the US region of Boulder, Colorado, is much lower than that the ~25%—55% carriage rates reported from other industrialised countries such as those in Europe (Bart et al., 2013; Krogsgaard et al., 2015; Scanlan et al., 2014).

Nine of the ten positive cases were adults whilst only one of the individual in the child/adolescent group was positive. This result is consistent with data from a recent study of adults and infants living in Maharashtra, India (Pandey et al., 2015) and Ireland (Scanlan et al., unpublished). In both instances, infants are *Blastocystis* negative compared to a high carriage rate in the adult population (Pandey et al., 2015)(Scanlan et al., unpublished). Conversely, in a recent study of 93 children living in the Senegal River Basin, 100% were *Blastocystis* positive (El Safadi et al., 2014). Interestingly, eight of the nine positives within the adult group were male. However, such a strong gender bias was not evident in other recent studies (AbuOdeh et al., 2016; Scanlan et al., 2014). The factors that account for both the disparity in *Blastocystis* prevalence between adult populations worldwide, and child/infant populations in different regions, including

different rates of carriage compared to contemporary adult populations is as yet unknown but could in part be linked to sanitation and water supply.

Each of the individuals positive for *Blastocystis* belonged to a different FU (i.e. no two individuals from the same FU carried *Blastocystis*), suggesting that direct human-human transmission within families is not a likely source of *Blastocystis* transmission for groups of individuals living in similar circumstances (with respect to degree of industrialisation, personal hygiene practices, access to sanitation, clean drinking water *etc.*).

A diversity of *Blastocystis* alleles were detected in our host population; ST1 (20%; allele 4), ST2 (30%; alleles 9 and 12) and ST3 (50%; alleles 34 and 36), which is consistent with data on the most prevalent STs detected in human populations (Alfellani et al., 2013). Interestingly ST4, which is considered rare outside of Europe (Alfellani et al., 2013) but has been detected in Australia (Nagel et al., 2014; Nagel et al., 2012), was not present in our dataset. However, this could be due to the low number of individuals that were positive for *Blastocystis* - further sampling of individuals from different regions is required to definitively establish the incidence and distribution of ST4 in the US. None of the dog samples tested positive, which supports a recent study showing that dogs may not be natural hosts of *Blastocystis* and therefore are an unlikely source of zoonosis (Wang et al., 2013).

Whilst our study provides essential information on the prevalence and genetic diversity of *Blastocystis* in a relatively understudied population, these data also have implications for our understanding of the epidemiology of *Blastocystis*. Although the role of *Blastocystis* in human disease is controversial, these results suggest that sharing an environment with a related or unrelated *Blastocystis* carrier is unlikely to pose a

transmission (disease) risk for those in similar living conditions. It is worth noting, however, that a recent study cited the incidence of *Blastocystis* in another family member as a predictor for *Blastocystis* infection (Anuar et al., 2013). However, the study in question was conducted on three tribal communities in Malaysia, many of whom lacked adequate water and sewage facilities (Anuar et al., 2013). Moreover, no genetic analysis of *Blastocystis* was performed so a direct investigation into possible human-human transmission was not conducted. The Malaysian study also identified the consumption of contaminated water as a primary risk factor in transmission; this may indicate that in families with more than one member positive for *Blastocystis* the incidence of *Blastocystis* may simply reflect their shared environment and consumption of unclean water rather than direct human-human transmission.

5. Conclusions

It is becoming increasingly clear that the prevalence of *Blastocystis* varies considerably between industrialised (and non-industrialised) populations and that source(s) of *Blastocystis* transmission to humans will likely vary depending on the population under study. The factors that underpin variation in prevalence are currently unknown but differences in global human living conditions, including sanitation levels, access to clean water and contact with animals that are host to *Blastocystis*, are likely factors in both prevalence rates and sources of transmission to humans. The continued publication of prevalence data derived from sensitive PCR-based approaches is central to increasing our understanding of *Blastocystis*' demographic and geographic distribution, potential pathogenicity, and mode of transmission to humans.

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Table 1. Overview of particpants, full details are provided in Supplementary Table 1.

Group	Mean age and range (years)	Male to Female Ratio	*Blastocystis* prevalence (%)
Adults, N = 101	34.8 (18-58)	1:1.1	8.9
Children/adolescents, N = 38	4.1 (0.5 -17)	1:1	2.6

Highlights:

- First PCR-based survey of *Blastocystis* in a US population using *Blastocystis* specific primers
- Prevalence of *Blastocystis* in this subset of the US population is comparatively low relative to other industrialised populations investigated to date
- *Blastocystis* subtype diversity is largely consistent with that previously reported from studies of European populations
- The distribution of *Blastocystis* within family units indicates that human-human transmission is unlikely to have occurred within families studied here.