*Parasitology*

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# The intestinal protist *Blastocystis* is not a common member of the healthy infant gut microbiota in a Westernized country (Ireland)

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## Abstract

Research into the gut microbiota of human infants is necessary in order to better understand how inter-species interactions and ecological succession shape the diversity of the gut microbiota, and in turn, how the specific composition of the gut microbiota impacts on host health both during infancy and in later years. *Blastocystis* is a ubiquitous intestinal protist that has been linked to a number of intestinal and extra-intestinal diseases. However, emerging data show that asymptomatic carriage is common and that *Blastocystis* is prevalent in the healthy adult gut microbiota. Nonetheless, little is known about the prevalence and diversity of this microorganism in the healthy infant gut, including when and how individuals become colonized by *Blastocystis*. Here, we surveyed the prevalence and diversity of *Blastocystis* in an infant population ($n = 59$) from an industrialized country (Ireland) using *Blastocystis*-specific primers at three or more time-points up to 24 months old. Only three infants were positive for *Blastocystis* (prevalence = 5%) and this was only noted for samples collected at month 24. This rate is comparatively low relative to previously reported prevalence rates in the contemporaneous adult population. These data suggest that infants in Westernized countries that are successfully colonized by *Blastocystis* most likely acquire this microorganism *via* horizontal transfer.

## Introduction

The importance of the gut microbiota to human physiological and immunological development, particularly during the early years of life, has led to efforts to characterize the composition and dynamics of the gut microbiota of humans from birth through infancy and into childhood (Arrieta *et al.* 2014; Backhed *et al.* 2015*a*; Rodriguez *et al.* 2015). This knowledge is required to identify different factors that influence gut microbiota composition and functionality through time and how changes in the gut microbiota impact on host health at various key stages of life (Subramanian *et al.* 2014; Frese and Mills, 2015).

Upon birth, the human gut is rapidly colonized by a diversity of microbes. Numerous studies have shown that the diversity of the neonate gut microbiota is affected by a range of factors including mode of delivery (vaginal or Caesarean section), whether the infant is breast or formula fed, and antibiotic use (Dominguez-Bello *et al.* 2010; Bokulich *et al.* 2016; Hill *et al.* 2017). Following birth, the bacterial population of the gut undergoes gradual succession and matures with key signature species and ecological networks observed at particular time-points (Backhed *et al.* 2015*a*). Between ~2 and 5 years of age, the bacterial population of the human gut begins to resemble that of an adult with respect to both diversity and richness (Koenig *et al.* 2011; Yatsunenko *et al.* 2012; Bokulich *et al.* 2016).

In addition to the bacterial fraction of the infant gut microbiota, researchers are now investigating patterns of colonization and diversity for other members of the gut microbiota, e.g. archaea, viruses and fungi, in order to determine the roles that such microorganisms play as drivers and/or moderators of intestinal health during early life (Lim *et al.* 2015; Ward *et al.* 2017). However, little is known about the prevalence and diversity of other potentially important microbes, such as protists, and what role, if any, they may play in infant health and disease. This dearth of knowledge extends to the microbial eukaryote *Blastocystis*, which is a common component of the human adult gut and is estimated to colonize over 1 billion people worldwide (Scanlan and Stensvold, 2013).

*Blastocystis* is a member of the Stramenopiles (or Heterokonta) branch of Eukarya (Silberman *et al.* 1996). This diverse assemblage of organisms encompasses both uni- and multi-cellular organisms such as diatoms, algae and oomycetes (Patterson, 1999). Currently, seventeen different *Blastocystis* subtypes (STs) or species have been described (Alfellani *et al.* 2013*a*) and, of these, nine have been recovered from human samples. Although a major focus in *Blastocystis* research is understanding the potential role of this microorganism in infection and intestinal disease, recent data have shown that it is a common component of the healthy adult gut microbiota (Scanlan *et al.* 2014; Beghini *et al.* 2017). Given that asymptomatic carriage is common, this suggests that *Blastocystis*' potential for pathogenicity is



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limited to certain genotypes and/or specific host–genotype and host–genotype–environment interactions (Scanlan and Stensvold, 2013).

*Blastocystis* prevalence rates vary significantly between different geographical regions (Alfellani *et al.* 2013), with the highest prevalence in a healthy European cohort published to date reported for a subset of the adult Irish population (Scanlan *et al.* 2014). Fifty-five per cent of adults in this study were positive for *Blastocystis* and, although within-host diversity was low, with the most individuals host to a single *Blastocystis* ST, 22% were host to two or more different STs (Scanlan *et al.* 2015). Although the factors responsible for the high prevalence rates of *Blastocystis* observed in the Irish population compared to other European countries are, as yet, unknown, variation in *Blastocystis* prevalence has been linked to a number of factors including levels of sanitation and exposure to contaminated water (Leelayoova *et al.* 2008; Speich *et al.* 2016). Following on from this study we wished to provide a more complete picture of the epidemiology of *Blastocystis* in the Irish population and also shed some light on how and when humans become colonized with *Blastocystis*. Accordingly, we investigated the prevalence and genetic diversity of *Blastocystis* in a cohort of healthy infants from a subset of the Irish population that had been sampled at a number of time-points up to 24 months of age.

## Material and methods

### *Overview of study and study participants*

The aim of our study was to provide longitudinal data on the prevalence and diversity of the intestinal protist *Blastocystis* in a healthy infant cohort from a Westernized European country (Ireland). The samples analysed were part of the INFANTMET study cohort (Hill *et al.* 2017) for which ethical approval was provided by the Cork University Hospital Research Ethics Committee (ethical approval reference: ECM (w) 07/02/2012). Fecal DNA samples were obtained from infants ($n = 59$) that were born either at full term ($n = 55$) or preterm ($n = 4$) and either *via* spontaneous vaginal delivery ($n = 30$) or Caesarean section ($n = 29$); see Table 1 for more details. Samples taken from week 1, week 8, 12 months and 24 months were analysed for all infants. Samples from 1 additional time-point (week 4) were analysed for three individuals that were positive for *Blastocystis*.

### Blastocystis *PCR and sequence analysis*

Genomic DNA was extracted from fecal samples as outlined previously (Hill *et al.* 2017). The primer set RD5 and BhRDr were used to amplify and sequence ~600 bp of the SSU rRNA gene for all samples according to a standard protocol (Scicluna *et al.* 2006; Scanlan *et al.* 2014). Positive PCR products were cleaned using the Qiagen QIAquick PCR clean up kit and sequenced (Source Bioscience, Ireland). Sequence data were trimmed and submitted to the online site http://pubmlst.org/Blastocystis/ to assign *Blastocystis* subtype and allele ID. Sequences were then aligned and analysed in MEGA4 (Tamura *et al.* 2007). Within-host *Blastocystis* diversity (so-called mixed infections) was also investigated using a recently developed ST-specific primer set as described elsewhere (Scanlan *et al.* 2015).

## Results and discussion

*Blastocystis* was detected in three of the 59 or 5% of the infant population tested, with all positives being 24-month samples. No positive PCR signals were detected for any of the samples taken from any infants at week 1, week 8 and 12 months including all samples from the three infants that were positive for *Blastocystis* at 24 months. Unfortunately, data relating to *Blastocystis* colonization of the mothers of the infants sampled here are not available. However, based on our previously published prevalence data (Scanlan *et al.* 2014, 2015) from a contemporaneous adult cohort living in the same region of Ireland, it is conceivable that >50% of them were positive. Based on this assumption, the absence of *Blastocystis* in all infants at the early time-points) indicates that *Blastocystis* was not acquired by any of these infants at birth and, in those individuals that were positive for *Blastocystis* at 24 months, it is likely that *Blastocystis* was acquired *via* horizontal transmission at some stage between years 1 and 2.

Each of the three positive PCR products could be assigned to one of three STs (ST2_allele_9, ST3_allele_31 and ST4_allele_42, respectively) using the online site http://pubmlst.org/Blastocystis/ (Jolley and Maiden, 2010; Stensvold *et al.* 2012). There was no evidence for multiple STs present within an individual host. Even though the number of positive hosts is low, the diversity of STs detected in this infant population is typical of those STs present in the healthy adult population (Scanlan *et al.* 2014).

Collectively, our data show that the prevalence of *Blastocystis* in this infant population is relatively low compared with an earlier study of the adult Irish population and that *Blastocystis* is likely to be acquired *via* horizontal rather than vertical transmission. Overall, these results are consistent with studies of *Blastocystis* prevalence rates in adults and infants in India. The first of these studies surveyed microbial eukaryotic diversity in mothers and their infants ($n = 4$) and found that whilst *Blastocystis* was detected by PCR and sequencing of DNAs pooled from the mother's samples, no *Blastocystis* signal was detected in the infant dataset (Pandey *et al.* 2012). A follow-up study reported a similar trend with *Blastocystis* prevalent in the adult population ($n = 100$, prevalence = 27%), and absent in the infant population, i.e. none of the 120 samples that had been obtained from thirty infants at various time-points between 7 days and 12 months old gave a positive result (Pandey *et al.* 2015). Similarly, a study of *Blastocystis* prevalence rates in families living in the US state of Colorado (Scanlan *et al.* 2016) found that though the overall prevalence rates for *Blastocystis* was low in this dataset, only one of 19 infants (5%) were positive for *Blastocystis*. This figure was lower than the adult population with nine out of 101 adults *Blastocystis* positive (9%). Interestingly, infants in the US dataset were aged between 0.5 months and 2 years and the positive sample was obtained from a 24-month old.

One of the possible explanations for the difference in *Blastocystis* prevalence rates between adult and infant populations may relate to differences in the diversity and composition of the gut bacteria in adults compared with children (Yatsunenko *et al.* 2012). Recent data have shown that the presence of *Blastocystis* in the adult gut microbiota is correlated with increased bacterial diversity and the presence of specific bacterial species (Andersen *et al.* 2015; Audebert *et al.* 2016). Given that the infant gut is much less diverse and differs in composition to the adult gut, it is possible that the conditions for successful colonization of *Blastocystis* (upon exposure) are only present once the infant's gut has matured and reached a more diverse community that develops as the child ages. To test this hypothesis a comparative analysis of the microbiota of large numbers of positive and negative *Blastocystis* samples is required. Unfortunately, this type of analysis is not possible here given the imbalance (very low numbers) of positives relative to negatives in our sample-set. Nonetheless, this proposed scenario is analogous to a recent observation that hydrogen-consuming microbes such as the *Desulfovibrio* spp. and *Methanobrevibacter smithii* are abundant in mothers yet virtually absent in their infants ($n = 98$)

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**Table 1.** Overview of study participants and results

| Subject code* | Fullterm/pre-term | Delivery type | Week 1 *Blastocystis* result | Week 8 *Blastocystis* result | 12 Month *Blastocystis* result | 24 Month *Blastocystis* result |
|---|---|---|---|---|---|---|
| 1 | FT | SVD | – | – | – | – |
| 2 | FT | LSCS | – | – | – | – |
| 3 | FT | LSCS | – | – | – | + |
| 4 | FT | SVD | – | – | – | – |
| 5 | FT | SVD/forceps | – | – | – | – |
| 6 | FT | LSCS | – | – | – | – |
| 7 | FT | SVD | – | – | – | – |
| 8 | FT | SVD | – | – | – | – |
| 9 | FT | SVD | – | – | – | – |
| 10 | FT | LSCS | – | – | – | – |
| 11 | FT | SVD | – | – | – | – |
| 12 | FT | LSCS | – | – | – | – |
| 13 | FT | LSCS | – | – | – | – |
| 14 | FT | SVD | – | – | – | – |
| 15 | FT | LSCS | – | – | – | – |
| 16 | FT | SVD | – | – | – | – |
| 17 | FT | SVD | – | – | – | – |
| 18 | FT | SVD | – | – | – | – |
| 19 | FT | SVD | – | – | – | – |
| 20 | FT | SVD | – | – | – | – |
| 21 | FT | LSCS | – | – | – | – |
| 22 | FT | Vacuum | – | – | – | – |
| 23 | FT | SVD | – | – | – | – |
| 24 | FT | SVD | – | – | – | – |
| 25 | FT | LSCS | – | – | – | – |
| 26 | FT | SVD | – | – | – | – |
| 27 | FT | LSCS | – | – | – | – |
| 28 | FT | LSCS | – | – | – | + |
| 29 | FT | LSCS | – | – | – | – |
| 30 | FT | LSCS | – | – | – | + |
| 31 | PT | LSCS | – | – | – | – |
| 32 | FT | SVD | – | – | – | – |
| 33 | FT | LSCS | – | – | – | – |
| 34 | FT | SVD | – | – | – | – |
| 35 | FT | SVD | – | – | – | – |
| 36 | FT | SVD | – | – | – | – |
| 37 | PT | LSCS | – | – | – | – |
| 38 | PT | LSCS | – | – | – | – |
| 39 | FT | LSCS | – | – | – | – |
| 40 | FT | SVD | – | – | – | – |
| 41 | FT | LSCS | – | – | – | – |
| 42 | FT | SVD | – | – | – | – |
| 43 | PT | LSCS | – | – | – | – |
| 44 | FT | SVD | – | – | – | – |
| 45 | FT | SVD | – | – | – | – |

(*Continued*)

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**Table 1.** (*Continued.*)

| Subject code* | Fullterm/pre-term | Delivery type | Week 1 *Blastocystis* result | Week 8 *Blastocystis* result | 12 Month *Blastocystis* result | 24 Month *Blastocystis* result |
|---|---|---|---|---|---|---|
| 46 | FT | LSCS | – | – | – | – |
| 47 | FT | SVD | – | – | – | – |
| 48 | FT | LSCS | – | – | – | – |
| 49 | FT | LSCS | – | – | – | – |
| 50 | FT | LSCS | – | – | – | – |
| 51 | FT | SVD | – | – | – | – |
| 52 | FT | LSCS | – | – | – | – |
| 53 | FT | SVD | – | – | – | – |
| 54 | FT | LSCS | – | – | – | – |
| 55 | FT | SVD | – | – | – | – |
| 56 | FT | LSCS | – | – | – | – |
| 57 | FT | LSCS | – | – | – | – |
| 58 | FT | SVD | – | – | – | – |
| 59 | FT | LSCS | – | – | – | – |

*Positive *Blastocystis* samples are highlighted in bold.
FT, fullterm; PT, pre-term; SVD, spontaneous vaginal delivery; LSCS, lower segment Caesarean section.

(with the exception of two 12-month infants that were colonized by *M. smithii*) (Backhed *et al.* 2015*b*). Here, the authors suggested that the presence of these microbes in the adult gut and their absence in the infant's gut was possibly due to increased fermentative capacity observed in the adult microbiota that creates a niche for microbes that can dispose of hydrogen as methane or other by-products.

Whilst emerging data highlight potential links between *Blastocystis* and other members of the gut microbiota as potential determinants of successful *Blastocystis* colonization, it is clearly necessary to consider exposure rates to this microorganism as another key factor that may explain differences in *Blastocystis* prevalence rates, particularly between different geographical regions. For example, a recent study of children in Nigeria showed that the proportion of 24-month-old infants that were positive for *Blastocystis* ($n = 7$, 40% prevalence) was much higher than the prevalence rates reported here and in the other referenced studies (Pandey *et al.* 2015; Scanlan *et al.* 2016). Although the number of infants sampled in the Nigerian study is low, these data highlight the importance of exposure which is likely to vary considerably between different geographical regions due to living and sanitation conditions, access to clean water and exposure to animals. Accordingly, we can expect to see variation between datasets based on geography for both infant and adult populations. Nonetheless, even if exposure rates can explain some of the variation in prevalence rates, age appears to be emerging as an important factor given that this longitudinal study of Nigerian infants and children also showed that *Blastocystis* prevalence rates increased significantly with increasing age; children aged four and over ($n = 192$) had prevalence rates of >80% (Poulsen *et al.* 2016).

## Conclusions

The continued provision of prevalence data on *Blastocystis* is contributing to our greater understanding of the ecology and epidemiology of this gut microbe. The almost complete absence of *Blastocystis* in healthy infant groups relative to its higher prevalence in adult populations reported here and elsewhere indicates that *Blastocystis* is not adapted to the naïve infant gut. Therefore, the successful colonization of *Blastocystis* in humans may require additional factors relating to the specific composition and diversity (maturity) of the gut microbiota.

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