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BLASTOHIT

Main results:

Using a combination of PCR and sequenced based analysis we have investigated the prevalence and genetic diversity of *Blastocystis* in a number of human groups of interest including healthy adults, healthy infants, and family units. We have shown that *Blastocystis* is highly prevalent in the healthy Irish adult population but is not a common feature of the healthy infant gut. We have also developed a novel primer set and PCR assay that will facilitate epidemiological surveys of *Blastocystis* in human and animal groups. This novel primer set has shown that mixed infections of *Blastocystis* are much more common than previously thought which may be relevant to disease groups. We are currently analyzing data to look at the link between *Blastocystis* and specific bacterial groups in the gut.

Opportunity / Benefit:

As a consequence of our studies, we have a new understanding of the prevalence of *Blastocystis* (a speculated emerging pathogen) in the Irish population. The development of a novel primer set allows the surveillance of this microorganism both in human cohorts and also potential sources of transmission to humans e.g. livestock, water, other humans groups etc. Moreover, any link between the presence/absence of *Blastocystis* and specific bacterial groups may result in novel beneficial ways to treat symptomatic infection by *Blastocystis*.

Collaborating Institutions:

UCC, APC, Wageningen University, University of Colorado. Statens Serum Institut, Copenhagen

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External collaborators: Prof. Paul O'Toole, University College Cork, Alimentary Pharmabiotic Centre
Dr Rune Stensvold, Copenhagen
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1. Project background:

It is now realised that the human intestinal microbiota is central to health and wellbeing. However, the vast amount of research into the human microbiome has focused primarily on the prokaryotic (bacterial) component of this complex microbial ecosystem and we understand virtually nothing of the eukaryotic species, such as the protists, that are known to play critical roles in structuring microbial communities in other natural ecosystems.

Blastocystis is the most common protist in the human intestinal tract (IT); current estimates indicate that it colonises up to 1 billion people worldwide. Crucially, *Blastocystis* is considered to be an emerging pathogen, but its role in human illness and disease is both controversial and unclear. In addition to humans, *Blastocystis* is found in a wide range of mammalian, non-mammalian and avian hosts, including livestock and poultry.

The key research goal of this project is to survey the prevalence and genetic diversity of *Blastocystis* in key groups of interest including healthy controls (adults and infants) to better understand the role of *Blastocystis* in human intestinal health and disease, how it is transmitted to humans and the link between *Blastocystis* incidence and other members of the human gut microbiota.

2. Questions addressed by the project:

- What is the prevalence of *Blastocystis* in healthy controls and individuals with IBS?
- Are particular *Blastocystis* genotypes associated with disease?
- Identify potential sources of *Blastocystis* transmission to humans?
- Is there a link between the presence/absence of *Blastocystis* and particular bacterial species (i.e. probiotic strains such as *Lactobacillus* spp. and *Bifidobacteria* spp.)

3. The experimental studies:

Study 1: Survey the prevalence and genetic diversity of *Blastocystis* in a cohort of adults using *Blastocystis* specific primers targeting the 18S rRNA gene following by sequence analysis.

Study 2: Development of a novel primer set to address the question of mixed infections and also to facilitate epidemiological surveys looking at potential sources of *Blastocystis* transmission to humans.

Study 3: Survey the prevalence and genetic diversity of *Blastocystis* in a cohort of family units in the US (in collaboration with Rob Knight) to look at human-human transmission

Study 4: Survey of *Blastocystis* incidence in healthy infants to investigate its prevalence. Further analysis to look at the relationship between bacterial diversity and *Blastocystis*. This study will also be linked to study 1 where we will also look at the presence/absence of *Blastocystis* and specific microbiota using bioinformatics analysis of 16S datasets for both studies and prevalence data.

4. Main results:

Study 1: We detected Blastocystis in 56% of healthy adults (n=105) surveyed. This figure is much higher than previously reported for an industrialised county (Ireland). We also detected a diversity of different subtypes (species) and *Blastocystis* was present in a subset of individuals sampled over a period of time between six and ten years indicating that it is capable of long-term host colonisation. Conclusions: These results show that *Blastocystis* is a common and diverse member of the healthy gut microbiota. The potential role of *Blastocystis* in intestinal disease may be linked to specific genotypes or host-genotype interactions.

Study 2: We developed and applied Blastocystis ST-specific PCRs for the investigation of the most common subtypes (STs) of *Blastocystis*, (ST1—ST4), to a healthy human cohort (n = 50). We detected mixed

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infections in 22% of cases, all of which had been identified as single ST infections in our earlier study (Study 1) using state-of-the-art methods. Certain STs occur predominantly as either single (ST3 and 4) or mixed infections (ST1), which may reflect inter alia transient colonisation patterns and/or co-operative or competitive interactions between different STs. We also performed comparative analysis with other primers that have been used extensively for ST-specific analysis found them unsuitable for detection of mixed and, in some cases, single ST infections. Conclusions: Collectively, our data shed new light on the diversity of *Blastocystis* within and between human hosts. Moreover, the development of these PCR assays will facilitate future work into the molecular epidemiology and significance of mixed infections in groups of interest, including health and disease cohorts, and also help identify sources of *Blastocystis* transmission to humans, including identifying potential animal and environmental reservoirs.

Study 3: Here we investigated the possibility of *Blastocystis* human-human transmission between healthy individuals (n = 139) using families units (n = 50) living in Boulder, Colorado as our sample-set. We detected a diversity of *Blastocystis* subtypes, however, the overall prevalence of *Blastocystis* was comparatively low (7%) with respect to other Western populations. Conclusions: Although we did not find any evidence of human-human transmission, given the world-wide variation in human living conditions and lifestyles we cannot rule out a role for this mode of transmission in other populations but instead propose that human-human transmission of *Blastocystis* is unlikely in human populations living in similar circumstances to those outlined here.

Study 4 and Study 5: Data is currently being analysed

5. Opportunity/Benefit:

The studies provided novel insight into the prevalence and genetic diversity of this potential pathogen in the Irish (and US) population and raises important questions as to if and how this microorganism may cause disease, and also how it is transmitted to humans. Furthermore, we have developed and optimized a number of sensitive PCR assays that allow for *Blastocystis* detection and genetic subtyping which will facilitate *Blastocystis* surveillance in other groups of interest including potential animal sources of transmission to humans. Also, the identification of any links between bacterial members of the gut microbiota such as probiotic species could result in novel anti-microbial intervention in individuals where *Blastocystis* carriage is an issue.

6. Dissemination:

The results of this project have been transferred through presentations both nationally and internationally and have also resulted in a number of peer-reviewed publications.

Main publications:

1. Scanlan PD (2012) *Blastocystis*: past pitfalls and future perspectives. Trends Parasitol 28: 327-334.
2. Scanlan PD, Stensvold CR, Rajilic-Stojanovic M, Heilig HG, De Vos WM, et al. (2014) The microbial eukaryote *Blastocystis* is a prevalent and diverse member of the healthy human gut microbiota. FEMS Microbiol Ecol 90: 326-330.
3. Scanlan PD, Stensvold CR, Cotter PD (2015) Development and Application of a *Blastocystis* Subtype-Specific PCR Assay Reveals that Mixed-Subtype Infections Are Common in a Healthy Human Population. Appl Environ Microbiol 81: 4071-4076.
4. Scanlan et al. Analysis of the prevalence and genetic diversity of *Blastocystis* within family units in the United States does not support a role for human-human transmission.(in preparation)
5. Scanlan et al. Low prevalence of *Blastocystis* in the infant gut is linked to bacterial diversity (in preparation)

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