Parasitology International 65 (2016) 763–771

Contents lists available at ScienceDirect

Parasitology International

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

Current status of *Blastocystis*: A personal view

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ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 10 May 2016
Accepted 27 May 2016
Available online 28 May 2016

Keywords:
Parasite
Gut
Stramenopiles
Public health
Clinical microbiology

ABSTRACT

Despite *Blastocystis* being one of the most widespread and prevalent intestinal eukaryotes, its role in health and disease remains elusive. DNA-based detection methods have led to a recognition that the organism is much more common than previously thought, at least in some geographic regions and some groups of individuals. Molecular methods have also enabled us to start categorizing the vast genetic heterogeneity that exists among *Blastocystis* isolates, wherein the key to potential differences in the clinical outcome of *Blastocystis* carriage may lie.
In this review we summarize some of the recent developments and advances in *Blastocystis* research, including updates on diagnostic methods, molecular epidemiology, genetic diversity, host specificity, clinical significance, taxonomy, and genomics. As we are now in the microbiome era, we also review some of the steps taken towards understanding the place of *Blastocystis* in the intestinal microbiota.

1. Introduction

It is now over 100 years since Alexeieff [1] first described the intestinal eukaryote *Blastocystis* but, despite the efforts of numerous researchers (especially in recent years), there are still many unknowns surrounding this organism. Most important of these is whether *Blastocystis* causes disease in humans. For every report linking *Blastocystis* with gastrointestinal or other symptoms there is another that finds no such link. There are a number of factors that have contributed to this apparent lack of progress and these will form the basis of this review. We would like to warn the reader at this early stage that we ourselves are convinced only that there are no definitive data yet available to resolve this issue.

2. Taxonomy and evolution

In culture, *Blastocystis* is generally spherical with no obvious surface features. When stained, the most common morphological form seen has a large central vacuole of unknown function and the cytoplasm with all the organelles is visible as a thin peripheral layer between the vacuole and the cell membrane (Fig. 1). While many morphological forms have been described, the significance of most is unclear, the boundaries between them are not discrete, and some may well represent degenerating forms [2]. We refer the reader to earlier reviews for more details [3–5]. The life-cycle is typical of most gut protists, with a resistant cyst form for transmission and a trophic form that divides by binary fission. More complex and alternative life-cycles have been described (discussed in [5]) but in our opinion there is no conclusive evidence for anything other than this simple two-stage life-cycle.

Blastocystis has a complicated taxonomic history. It has been viewed as a fungus, a sporozoan and even the cyst of another organism at various points in its history, until 20 years ago [6] when it was finally placed among the Stramenopiles. This is one of the major groups of eukaryotes [7], but one that, to date, contains only a single other human-infective eukaryote, *Pythium*. *Blastocystis* has none of the typical features of a stramenopile, which is in part why identifying its correct relationships took so long.

Since its classification as a Stramenopile further data have emerged regarding the closest relatives of *Blastocystis*. These turn out to be poorly known flagellated or ciliate-like organisms that live in vertebrate intestines. While most Stramenopiles are free-living and aerobes, *Blastocystis* and its relatives are gut-living and anaerobes, although they do have mitochondrion-like organelles (see later). *Blastocystis* is related specifically to the Proteromonadidae and Slopalinida [8], but these cannot be considered close relatives. However, it seems likely that the common ancestor of these groups of organisms was already living in a gut and an anaerobe.

The simple spherical morphology of *Blastocystis* mentioned above applies to all members of this genus. This means that morphology is of no use in defining species. Traditionally, *Blastocystis* species have been defined by the identity of their host, with all human *Blastocystis* being assigned to *Blastocystis hominis*. However, even before DNA sequences identified *Blastocystis* as a Stramenopile it had become clear that significant heterogeneity existed among human *Blastocystis*. Using serology, isoenzymes and karyotyping, human *Blastocystis* were being divided

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http://dx.doi.org/10.1016/j.parint.2016.05.015
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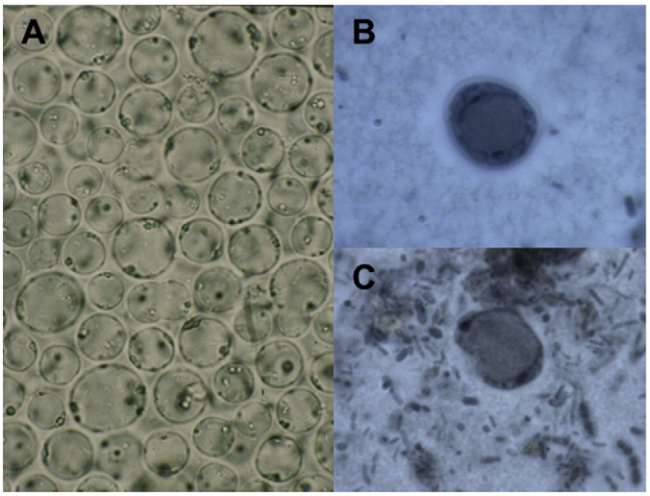

Fig. 1. Light microscopy images of *Blastocystis*. A. *Blastocystis* in culture. Using Robinson's and other media [29], *Blastocystis* often reaches high density in xenic culture. This stage is typically reported as 'vacuolar' due to the large central region of uncertain function. Organelles are seen as 'dots' along the periphery of the cell. B and C. *Blastocystis* in fecal smears, stained using iron-hematoxylin. Prominent nuclei are seen in the periphery of the cells as the most conspicuous morphological hallmark, along with the large central 'void'. Other organelles can be discerned as smaller peripheral 'dots', which will include the mitochondrion-like organelles, etc. However, these can only be positively identified by transmission electron microscopy. Images courtesy of John Williams (A) and Claire Rogers (B, C), Diagnostic Parasitology Laboratory, London School of Hygiene and Tropical Medicine.

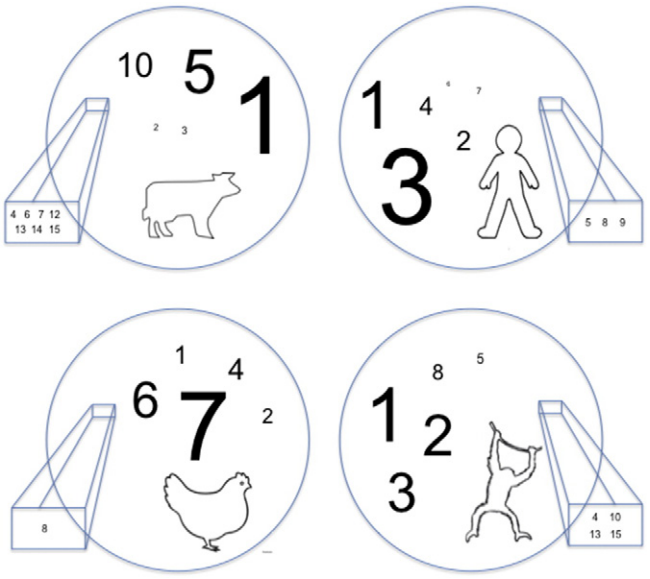

Fig. 2. Host range and relative prevalence of *Blastocystis* subtypes. In this schematic, the range of subtypes reported for four major host groups (humans, non-human primates, ungulates and birds) is shown. In the circle, the numbers are those of the most common subtypes found in the respective host, with the integer font size proportional to its prevalence. Numbers in the magnified boxes represent those subtypes that each constitute less than 5% of the total samples subtyped to date. Derived from the numbers presented in reference [10]. As an indication, prevalence figures for STs 1–4 in humans are 28.0%, 10.9%, 44.4% and 10.0% respectively.

into subgroups [4], and this picture of variation was reinforced by direct and indirect DNA sequence analyses [9]. Subsequent data have only added to the diversity and have refined our understanding of this genus.

Analyses of human *Blastocystis* by different researchers always resulted in the detection of variation, but each group came up with its own nomenclature for the groupings it identified. To resolve this confusion a consensus terminology was agreed [9] and this classification of human *Blastocystis* into numbered subtypes has simplified communication among workers in this field. At the time of the consensus two things were clear: 1. that humans were host to a number of distinct small subunit rRNA gene (SSU-rDNA)-based subtypes of *Blastocystis*, and 2. that most of these subtypes were also found in other mammalian or avian hosts. This meant the host-linked binomial species names were untenable, as the same organism was being called by multiple names. For example, one grouping of *Blastocystis hominis* proved to be genetically indistinguishable from *Blastocystis ratti*; both are now known as *Blastocystis* subtype 4 (ST4).

The current taxonomy of *Blastocystis* follows a distinct structure for mammal and bird organisms compared to all others [10]. The mammalian/avian *Blastocystis* are subdivided into seventeen subtypes (STs), nine of which (ST1–ST9) have been found in humans. There is host range overlap observed for many of these organisms (Fig. 2). *Blastocystis* from reptiles, amphibia and invertebrates retain Linnean binomial names for the most part. This is largely because little investigation of diversity and host range of these *Blastocystis* has been undertaken to date and so the same impetus to change the nomenclature has not existed. Whether a similar situation involving broad host-range and large genetic diversity will be uncovered in those organisms remains to be seen; it seems likely, and therefore the nomenclature of *Blastocystis* in those hosts may require a similar solution.

3. Genetic diversity and host specificity

Subtypes of *Blastocystis* are discrete and no intermediate variants have been uncovered to date despite extensive sampling from around the world. However, many host species remain to be sampled, so this picture may change. Guidance on how and when to define a new subtype has been published [11]. The recommendation is that a minimum of 5% sequence divergence from the SSU-rDNA of known subtypes is required before defining a new subtype is appropriate. One of the reasons for establishing this boundary is that *Blastocystis* subtypes are often assigned based on the sequence with the closest similarity in sequence database searches, without taking into account the degree of similarity. So a sequence that actually represents a new subtype may be assigned to an existing subtype. This misattribution has been a problem in some existing cases, for example ST13, as discussed in reference [10]. Unfortunately, information attached to entries in GenBank databases are rarely corrected and this can result in misidentifications being propagated forward in the literature.

The 5% level of divergence to define a new subtype was chosen in part because variation within subtypes can also be substantial, up to at least 3% [11]. Therefore a single 'outlier' sequence that appears to be distinct and potentially a new subtype could eventually merge into an adjacent subtype as more sequences become available. Only as more subtyping data accumulate will the validity of this arbitrary threshold be tested. Note that 5% divergence is the recommendation for establishing new subtypes, where sampling is likely to be limited. The divergence between some existing subtypes (for example, ST6 and ST9) is actually less than 5%. However, sampling is sufficient to give us confidence that these are indeed distinct lineages rather than variants of the same subtype. In other words, 5% divergence has been chosen as quite a stringent criterion and more data may lead to the revision of new subtype definitions in the future.

As mentioned earlier, nine distinct subtypes have been found in humans (Fig. 2). However 95% of human infections sampled belong to one of just four of these subtypes (STs 1–4; [12]) and only one of the human subtypes has not yet been found in another host: ST9 can claim (at present) to be restricted to humans. The four most common STs in humans have also been detected in other hosts. Most frequently these hosts are other primates, but they have also been found in various hoofed mammals, rodents and even birds [10]. Conversely, the rarer subtypes in humans (STs 5–8) are more commonly found in other hosts: ST5 in hoofed animals, STs 6 and 7 in birds, and ST8 in non-