Genotype Classification of *Blastocystis hominis* Isolates from Subjects with and without Irritable Bowel Syndrome by Polymerase Chain Reaction

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Introduction and Rationale

Blastocystis spp. are unicellular parasites that inhabit the lower GIT of humans and many animals. Its pathogenic potential is still a debated issue due to the fact that it is the most frequently encountered parasite in healthy individuals as well as in patients with GIT symptoms. Several studies showed that *Blastocystis* infection can be associated with irritable bowel syndrome (IBS), suggesting a possible role for the parasite in the IBS etiology. Molecular studies revealed high genetic diversity among *Blastocystis spp*., with the genus comprising at least 13 subtypes, nine of which (subtypes 1–9) could infect humans. Thus, it was hypothesized that certain subtypes may contribute to its pathogenic potential and to the clinical outcome of infection.

Study Objectives

(1)Identify subtypes of *Blastocystis* clinical isolates obtained from different patient groups; IBS, non-IBS acutely GIT symptomatic patients and asymptomatic subjects.

(2)Evaluate the infectivity and pathogenicity of detected subtypes from each group in experimentally infected rats.

Subjects and Methods

Study type: A case control study.

Subjects

-Three groups (19 *Blastocystis*-infected subjects/group)
- Group (I): IBS patients.
- Group (II): Non-IBS acutely GIT symptomatic patients.
- Group (III): Asymptomatic subjects.

-Only positive samples for *Blastocystis* without associated pathogenic parasites or bacteria were included in the study.

Methods

-*In vitro* propagation of *Blastocystis* into Jones' medium.

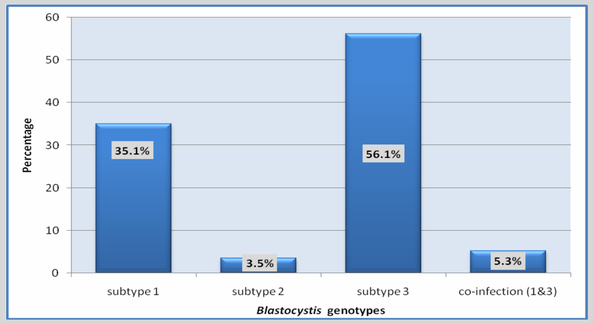
-Genotyping of *Blastocystis* clinical isolates using PCR with seven different sequence-tagged site primers.

- Assessment of infectivity and pathogenicity of *Blastocystis* subtypes in experimentally infected rats by oral inoculation of rats with 5×10^6 culture forms of a representing isolate for each of the detected subtypes in each group(4 rats/subtype). Rats were screened for infection daily for 1 week post-inoculation.Two weeks post-infection, rats were sacrificed and their large intestine was assessed grossly and histopathologically using H&E staining.

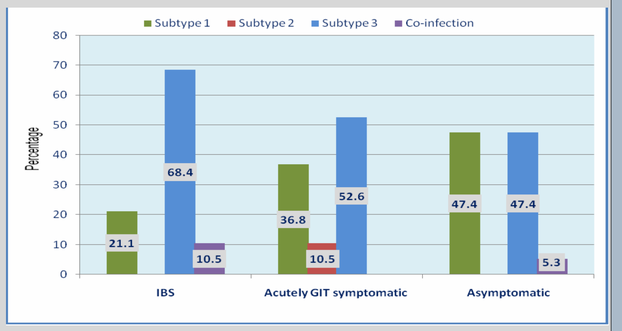
Results

Genotyping of *Blastocystis* clinical isolates:

Prevalence of *Blastocystis* genotypes among studied population



Prevalence of *Blastocystis* genotypes among studied groups



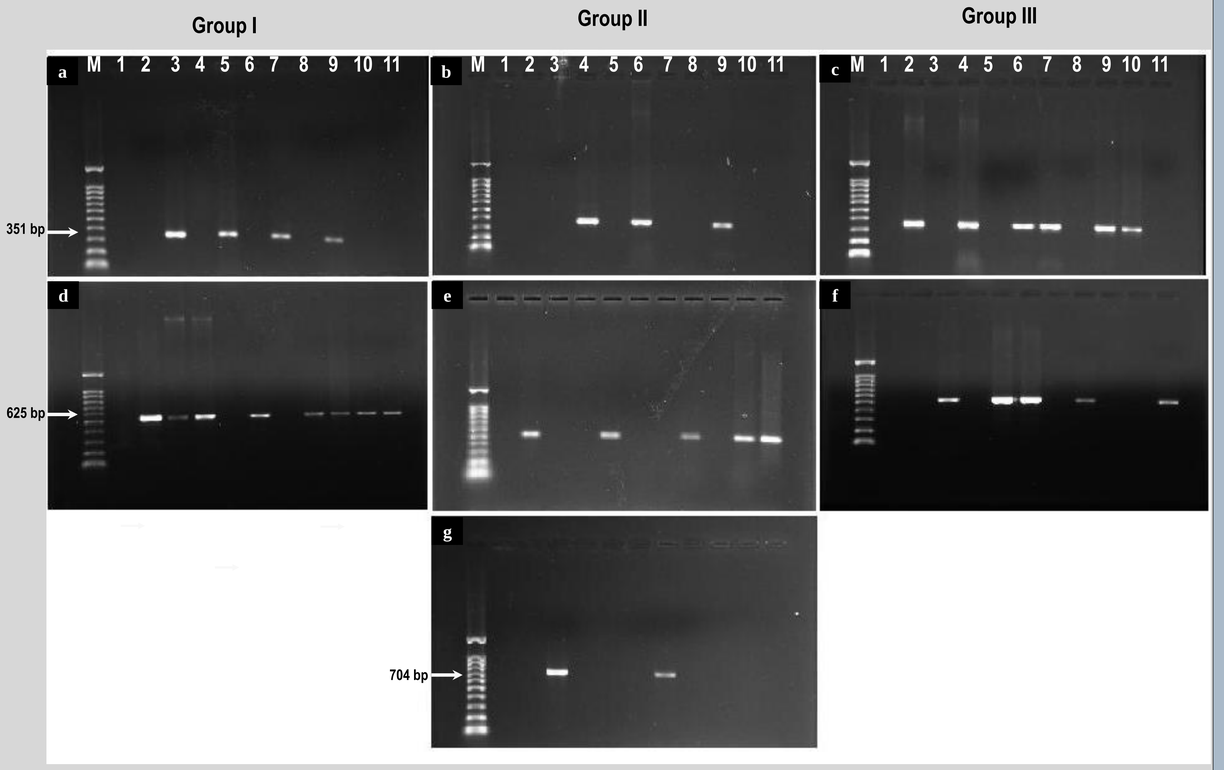


Fig. (1) Example of PCR amplification of *Blastocystis* isolates from G I (IBS group), G II (acutely GIT symptomatic group) G III (asymptomatic group).

M is the ladder DNA at 100 bp. Subtype 1 (351 bp) in fig. a, b and c, Subtype 3 (526 bp) in fig. d, e and f and subtype 2 (704 bp) in fig. g. Co-infection with ST 1 and 3 in G I (lane 3 and 9 fig. a and d) and G III (lane 6 fig. c and f).

Results cont.

All detected *Blastocystis* subtypes, used in animal inoculation, were found to be infectious to rats. The severity of pathological changes detected in experimentally infected rats is not dependent on the inoculated *Blastocystis* subtype, but rather on the clinical presentation of patients that *Blastocystis* parasites were isolated from, where isolates from acutely GIT symptomatic group showed severe inflammatory changes (Fig. 2), while isolates from IBS and asymptomatic groups showed mild inflammatory changes.

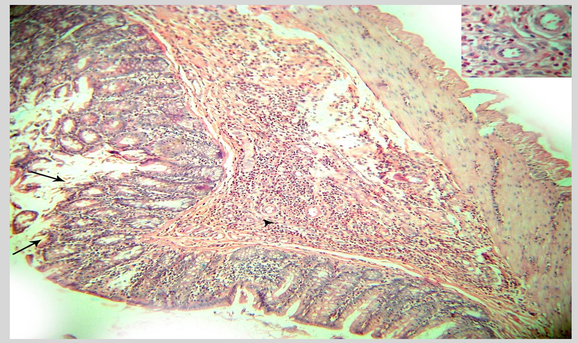


Fig. (2) Section in caecum showing severe inflammatory reaction with areas of mucosal sloughing [arrows] (H&E ×100).

Conclusion

1. Being the most predominant, subtype 3 could be the only subtype of human origin with high host specificity.

2. Virulence or pathogenic potential of *Blastocystis* could be due to intra-subtype variation or that pathogenic and non-pathogenic strains may exist in different isolates of the same genotype, which could explain the detection of some subtypes in both disease and control groups.

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