Reviewer’s report

Title: Diagnosis and subtype analysis of Blastocystis spp. in 442 patients in a hospital setting The Netherlands.

Version: 2 Date: 26 June 2013

Reviewer: C. Graham Clark

Reviewer’s report:

The authors have made a good job of addressing the majority of the reviewers’ comments. A few things still need to be clarified or corrected.

Minor Essential revisions

One thing I noticed this time is inconsistency in the use of Blastocystis spp., Blastocystis sp. and just Blastocystis to describe the organisms. 'spp.' implies there is more than one species but as there is no consensus on species names for human Blastocystis this is probably not the correct term to use in most contexts. 'sp.' is the term suggested by Stensvold et al. in their 2007 "consensus" paper, but the authors only use this once (page 10, line 19). The Background paragraph of the Abstract is probably confusing for anyone not already familiar with Blastocystis diversity and so I would suggest saying : "Blastocystis in humans consists of at least 9 genetic subtypes."

Personally I would suggest calling the organism just 'Blastocystis' in all cases in this manuscript, except perhaps the first usage and the title, where it should be Blastocystis sp. Certainly the terminology should be more consistent than it is at present.

The manuscript title is missing either a comma or the word 'in' after 'setting'.

p5, line 7 - 'differences in'
p5, line 9 - 'food, with sources'
p5, line 12 - 'as an opportunistic'
p5, line 15 - 'the number of parasites present,'
p5, lines 17-18 - there are now 17 subtypes described (Alfellani et al., Protist, in press) but it is not necessary to update to this. However there are a lot of named species from reptiles so the statement as written is not correct. I suggest rewording it to say: 'Currently, 14 subtypes of Blastocystis have been described in mammals based on sequence analysis, of which 9 have been reported in humans.'
p7, line 9 - '-formalin), while the tube'
p7, line 12 - 'protozoa' is misspelled
p7, line 14 - '(cysts and eggs of)'


p7, line 20 - the meaning of the word 'surface' is unclear here - should it be 'slide'?
p7, line 22 - 12.5x
p7, line 23 - 'The diagnostic criterion'
p7, line 24 - 'in either of the two'
p8, line 10 - I am not sure what is meant by 'an appropriate suspension'. Could a v:v ratio or something similar be given here?
p8, line 20/21 - the volume of DNA added is not useful without the total volume of the reaction being given. Also, how much of the product was loaded onto the gel? Some must have been retained for sequencing I expect.
p9, line 5 - 'for which there was no PCR'
p10, line 4 - 'TFT sets from 442 patients'
p10, line 13 - 'in either of the two'
p10, lines 21-23 - are you suggesting that this was actually Blastocystis but that the primer binding region was divergent? It is unclear.
p10, lines 24/25 - Since microscopy relied on vacuolar forms of Blastocystis, is it not possible that only cysts were present in these samples?
p11, line 15 - 'was not due to inefficient DNA extraction'
p11, line 21 - 'explained' is misspelled
p12, lines 8 and 11 - 'significant correlation'
p14, line 1 - 'high diagnostic sensitivity for Blastocystis'
p14, line 11 - what is meant by 'highly diverse' here? It could mean several things depending on how it is interpreted.
p14, line 12 - 'found as the most common'
p14, line 14 - 'Various studies have identified'
p14, lines 15-17 - it is a bit misleading to say 'most other reports' and then cite only two - perhaps cite a recent paper that summarizes all the geographic reports instead (eg ref 24)? Reports from the UK and Sweden all have ST4 as the 2nd most common subtype whereas it is the 4th in this study.
p14, line 20 - 'exposure to cysts'
p15, line 15 - I am unclear what is meant by 'special interests' here.
p15, line 17 - 'studies on pathogenicity'
p15, line 18/19 - 'especially the importance' and 'identification of an effective'

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.