Reviewer's report

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

Version: 1 Date: 18 March 2013

Reviewer: C. Graham Clark

Reviewer's report:

This is a fairly straightforward manuscript describing carefully performed work that generates significant new data on subtype prevalence of Blastocystis, extending the geographic coverage to The Netherlands. Most of my discretionary revisions relate to information I feel is missing from the paper, but since most of it presumably exists I think the ms could be improved by including slightly more detail.

Minor compulsory Revisions:

1. Some of the citations are out of date. For example there have been 14 Blastocystis subtypes for some time. ST14 was published six months ago and available 3 months before that (Fayer et al., Parasitology Research 2012; page 5 line 17). Also page 13 line 17 fails to cite recent European studies by Poirier in France (2011), Forsell in Sweden (2012) and Alfellani in the UK (2013) as well as other Danish studies that have a significantly higher ST4 prevalence than detected here. A more complete discussion of relative prevalence related to geography would also be helpful.

2. Blastocystis is not always italicised; spp. is not always followed by a period/full stop; Microsporidia should not be italicized.

3. Page 5 line 3 – here ‘faecal’ is used but on line 8 ‘fecal’ is used. Except for ‘Triple Faeces Test’ ‘faec’ is only used in ‘defaecation’ – please be consistent in usage.

4. Line 22 – there is no information on how the amplicons were purified, whether they were sequenced using the amplification primer(s), and whether one or both strands were sequenced.

5. References – in addition to the ones that seem to be missing mentioned earlier, reference 1, 17 and 23 are incomplete (no volume/pages), reference 12 lacks the final part of the citation (should be Euro Surveill. 16(24):pii=19891 ), reference 14 has an author name incomplete (Delgado-Viscogliosi), in reference 18 Blastocystis is not capitalized, and references 6 and17 have the doi information included but none of the others do.

6. Finally, in Table 1, the Total in the final column should be 4 not 1.

Discretionary Revisions:

1. Page 3 line 9 – I think this is slightly misleading. As written it implies that the
microscopy performed on the three samples was the same, which it was not, and seems to indicate that PCR was performed on a fourth sample. I believe it would be better worded as follows: Advanced microscopy on two samples and sequence confirmed PCR on a third sample from the same individual were used...

2. Line 12 – I believe it would be clearer if it said: …gold standard of combined sequence-confirmed PCR and positive advanced microscopy...

3. Page 6 line 24 – how was ‘2 clear vacuolar forms’ chosen as the criterion?

4. Page 7 line 9 – it would be worth specifying here that DNA was extracted and tested for ALL day 2 samples from each patient, not just microscopy positive ones. This eventually becomes clear from the text but it would be simpler to specify here.

5. Page 8 line 2 – how many sequences appeared to be mixed and were these all mixed Blastocystis subtypes or Blastocystis plus a non-specific sequence? Some comment on whether intra-subtype variation was seen would be useful. Are the sequences going to be deposited in GenBank?

6. Page 9 line12ff – sample 2 was also examined by microscopy after FECT. It would be useful to state how often Blastocystis was detected since FECT has been reported to be less sensitive for Blastocystis detection. If possible also report on whether subtypes were equally affected. Also, do the authors have any information on infection intensity being correlated with subtype? On page 10 line 18 it is mentioned that this information was recorded for at least two samples. The literature suggests that intensity may be correlated with symptoms, although I am not convinced, and if the authors have information that could contribute to resolving this issue it would be welcome.

7. Page 10 line13ff – there are other possible explanations for the differential sensitivity of microscopy and PCR on some samples. 1. The microscopy +ve cells detected may not actually have been Blastocystis; 2. Since microscopy and PCR were performed on different samples, the PCR –ve sample may actually also have been microscopy –ve; 3. Evidence suggests that certain Blastocystis PCR primers do not amplify all subtypes equally.

8. Line 22 – which subtype was identified from the weak band?

9. Page 11 line 6 – was there any correlation between subtype and age?

10. Page 13 lines 18-19 – I do not understand the link being made between different relative prevalence and different transmission cycles. Are the authors suggesting zoonotic transmission of ST3, for example? Alternatively prevalence could be linked to variable cyst numbers produced by different subtypes, or differential survival of subtypes in the environment, or different infectious doses being needed. None of these are really to do with the transmission cycle.

11. Lines 19-20 – is there any information on these patients that might link them to birds?

**Level of interest:** An article whose findings are important to those with closely
related research interests

**Quality of written English:** Acceptable

**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