Reviewer's report

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

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**Reviewer:** Hisao Yoshikawa

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This manuscript reports on the subtyping of human Blastocystis detected from clinical fecal samples in Netherlands. For the detection of Blastocystis, the authors had applied SAF preservation for the examination of light microscopy and PCR subtyping with eluted DNA by the MagNA pure LC isolation kit (Roche).

However, the previous study showed microscopic diagnosis of SAF-fixed stool samples for Blastocystis resulted in poor diagnosis among all five European reference laboratories (Utzinger et al. 2010 Clin. Microbiol. Infect. 16:267). Conversely, MagNA pure LC isolation kit also reported as low rate of detectability of Blastocystis at DNA level compared to other kits (Yoshikawa et al. 2012 Parasitol. Res. 109:1045) and three Blastocystis-positive samples among 21 fecal samples could not detected by PCR with DNA samples extracted by MagNA pure LC isolation kit (Morris et al. 2009 Parasitol. Res. 104:341).

These results indicate that SAF preservation and MagNA pure LC isolation kit are limited for detection of Blastocystis.

Therefore, this study could not detect real prevalence of Blastocystis in the human fecal samples in Netherlands based on SAF-preserved samples and PCR subtyping with the extracted with MagNA pure LC isolation kit. It is also difficult to evaluate the results of relationship between specific subtype(s) and patient history based on the inappropriate methodologies.

The authors should use more sensitive methodologies for the diagnosis of human Blastocystis, such as short-term in vitro culture or more sensitive kit for extraction of the DNA from fecal samples.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Not suitable for publication unless extensively edited

**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