Reviewer's report

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

Version: 1 Date: 23 March 2013

Reviewer: Eric Viscogliosi

Reviewer's report:

Blastocystis is an anaerobic parasite that inhabits the intestinal tract of humans and a wide range of animals. To date, Blastocystis is the most common intestinal parasite found in human feces and its prevalence is by far higher than those of other intestinal protozoan parasites such as Giardia, Entamoeba and Cryptosporidium. Its prevalence usually exceeds 5% in the general population of developed countries, can reach 30 to 60 % in developing countries, and largely exceeds 40% in individuals with chronic gastrointestinal illness such as irritable bowel syndrome (IBS). This parasite is also frequently found in immunocompromised individuals and a higher risk of Blastocystis infection has been found in humans with close animal contact emphasizing its zoonotic potential. It also exhibits a large genetic diversity that could be correlated with the differential pathogenic power of the 13 subtypes (STs) identified so far. Accumulating recent studies shed new light on the pathogenic power of this parasite suggesting that Blastocystis infection is associated with a variety of gastrointestinal disorders, may play a significant role in IBS, and may be linked with skin disorders.

Therefore, the exploration of the genetic diversity of Blastocystis isolates in human populations is of special interest for understanding the biology and assessing the public health significance of this parasite. It also permits characterization of the transmission dynamics and pathogenicity of the different Blastocystis STs. To my knowledge, the authors have performed in the present study including a large number of patients the first epidemiological survey of Blastocystis isolates in The Netherlands. They show the high prevalence of the parasite in their hospital-populations of patients (24%) and acquired the first data regarding the prevalence of different STs. As in most countries around the world, ST3 was predominant followed by STs 1, 2 and 4. These data have been compared to those obtained from other hospital populations in different countries. In addition, the authors have shown the high sensitivity of stool examination with microscopy which is almost identical to that of PCR if performed by technicians having extensive experience in parasitological diagnosis. Interestingly, the authors propose a “standardized” protocol for routine microscopic examination of stools with the use of the Triple Faeces Test.

This work is an interesting and useful contribution to our understanding of genetic diversity of Blastocystis. This study also proposes a protocol to improve the detection of this parasite by microscopic observation. Globally the manuscript is clearly and concisely written and no superfluous tables are present. Thus I think
that the paper should be considered for publication but I would like to see some minor essential revisions addressed first:

Minor essential revisions

1). In the background part of the manuscript, the authors should include more recent publications in the field. For instance, regarding the association between Blastocystis infection and IBS, the reviews by Boorom et al. (2008), Yakoob et al. (2010), Dogruman-Al et al. (2010) and Poirier et al. (2012) should be included. This is also the case for the study by Tan et al. (2009) regarding Blastocystis infection in immunocompromised patients. Concerning the genetic diversity of Blastocystis, the reference Parkar et al. (2010) should be definitely added. Concerning the transmission of the parasite, the studies by Eroglu et al. (2010) and Leelayoova et al. (2008) should be added. In addition, the authors indicate in the text that “recent studies suggest certain molecular methods to have highest sensitivities”. However recent studies describing the development of real-time PCR assays for sensitive and specific detection of Blastocystis by Stensvold et al. (2012) and Poirier et al. (2011) are not mentioned. This part of the manuscript must be reconsidered.

2) It seems that the sequences obtained in this study were not submitted to the Genbank since accession numbers are not mentioned in the text. GenBank accession numbers have to be supplied because the corresponding sequences could be used in further epidemiological or phylogenetic studies.

3) By experience I remain skeptical regarding the determination of predominant ST for sequences containing a mixed signal due to co-infections. Is that the authors are confident of the sequences obtained in these cases? What is the percentage of cases of co-infection? Is that the sequences obtained in the case of co-infections were included in the statistical analysis? They should be logically excluded from these analyzes. Globally, the authors should clarify these points.

4) Table 1: the percentages of each ST should be included in this table in brackets next to the corresponding number of isolates.

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