Reviewer's report

Title: Children of Senegal River Basin show the highest prevalence of Blastocystis sp. ever observed worldwide

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Reviewer: Ken Boorom

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The paper presents a prevalence study of Blastocystis infection in Senagelese children, with subtyping information, along with an analysis of potential relationships between infection, subtypes, and symptoms.

As few studies exist of Blastocystis in hyper-endemic environments, especially with subtyping, the paper's topic is of interest.

A few items of concern appear below:

1. (SELECTION CRITERIA) The paper does not indicate the selection criteria used for the 93 children. Although this may appear in the clinical trials referenced, it should be included in this paper (example: "Children were recruited through healthcare providers, who were asked to provide written material to all healthcare seeking patients between January 1, 2012 and March 30, 2012")

Enough information should be provided to understand any possible influences the selection criteria might have on the study's results. For example, if patients are recruited from healthcare seeking patients, then patients who are more prone to illness, or patients from families with better access to healthcare might be over-represented.

2) (NEGATIVE CONTROLS) The study notes that 100% of the DNA samples negative by non-q PCR tested positive by qPCR, using more powerful amplification techniques. But the study does not describe the use of negative controls, preferably a sample known to be Blastocystis-negative which was subjected to the same DNA extraction method.

The concern here is the possibility that the qPCR-positive results may be artifacts related to contamination, either from other samples, reagents from suppliers, or environmental sources (dust, etc.). qPCR is extraordinarily sensitive - for clinical work, Roche has required that PCR amplification be performed in a lab physically separated from the lab where DNA is extracted, for example. In one lab I've worked with, 10% of the samples were dedicated to negative controls (saline), and at one point, these began testing positive for Blastocystis, necessitating a shutdown of the facility for deep cleaning.

Recent studies have found that reagents from suppliers have enough DNA contamination to test positive using highly sensitive amplification techniques (i.e. Smith Retrovirology 2010, 7:112, Contamination of clinical specimens with...
3. Lines 309-310 indicate that the prevalence of Blastocystis was higher in symptomatic children:
   a. The authors should evaluate a 2x2 contingency matrix, preferably with Fisher’s exact test, and report the p-value for this relationship.
   b. The statement is confusing, as the paper indicated that 100% of the participants were infected.

4 (METHOD FOR DETERMINING SYMPTOMS): The authors should provide more information on the way symptom information was collected for those interested in repeating the study with other groups. For example, were children asked to list GI symptoms? Or were they asked about each symptom individually. If a written form was used, please include a copy of the form.

(5) It is difficult to discern individual rows of data in Table 1 - extra spacing or borders between adjacent rows would be most helpful.

(6) Lines 333-335: "coexistence of pathogenic and nonpathogenic 335 isolates of ST1, ST2, and ST3 among our Senegalese cohort":

I believe there is a logical fallacy in this statement, because the authors are neglecting to consider the influence of host factors.

For example, about 35% of Plasmodium falciparum infections are asymptomatic due to host factors (Sutcliffe, Reduced Risk of Malaria Parasitemia Following Household Screening and Treatment: A Cross-Sectional and Longitudinal Cohort Study, PLOS-One). Although there are asymptomatic infections of Plasmodium falciparum, as yet, we don't have compelling evidence of a non-pathogenic strain of P. falciparum.

If we divide P. falciparum into two lineages PL1 and PL2, which have equal virulence, and infect 50 individuals with each type, we would find about 35 asymptomatic cases, split evenly between PL1 and PL2, and 65 symptomatic cases similarly split.

By the author's logic, the PL1 and PL2 in the asymptomatic cases would have to be reclassified as non-pathogenic strains of P. falciparum within the PL1 and PL2 subgroups, only because they occur in individuals without symptoms.

A similar analysis holds for Giardia assemblages A and B. A typical study might show that 50% of individuals infected with Giardia Assemblage A or B show symptoms. But researchers generally have not concluded that this must prove the existence of non-pathogenic sub-strains within Assemblage A and B, because of the influence of host factors. Similarly, 40-60% of individuals infected with Hepatitis C show symptoms, which is believed to be due to host factors, rather than a non-pathogenic Hepatitis C.
A prevalence of nn% was reported in Australian zoo handlers. Suggested: suggests
Remove: Thereby,
Comma after handlers
Suggest removing word "other"

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

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