Reviewer's report

Title: Prevalence and Genetic Characterization of Cryptosporidium, Enterocytozoon, Giardia and Cyclospora in Diarrheal Outpatients in China

Version: 2 Date: 16 November 2013

Reviewer: Wilson Chan

Reviewer's report:

Thank you very much for your manuscript.

Major compulsory revisions:

The most major concern that exists is whether or not the detection of Cryptosporidium, Enterocytozoon, and Giardia actually represent etiologic agents of the patients' diarrhea. The study is concerning as there has been no testing of healthy, case-matched controls; as such, it is impossible to evaluate whether the findings are associated with disease. For example, as the authors themselves point out, there is ample C. andersoni in source and tap water in Shanghai; it may well be that even healthy people have detectable DNA of C. andersoni in their stool as a result of water ingestion.

In order to make the claim that these agents were identified as important etiologic agents, as the authors do in the discussion, it would be absolutely critical to include a set of case-matched controls, with sufficient statistical powering to detect a difference between the two groups.

Furthermore, it would be helpful to correlate the PCR results with standard diagnosis of Cryptosporidium, Enterocytozoon, Giardia, and Cyclospora. While PCR is a more sensitive method, correlation with standard parasitological methods would strengthen the validity of the PCR results.

It is also not clear if the PCR testing occurred over the entire study collection period, or was performed in one batch after all 252 specimens were collected. Especially in the latter case, it would be important to have controls during the testing to rule out cross-contamination. This was likely done, but explicitly outlining this would again strengthen the validity of the results.

At the current time, the lack of testing of healthy controls and the reliance on molecular methods alone do not allow the results to support the conclusions as stated.

Minor Essential Revisions:

Minor typographical and language errors, but these are easily remedied. In the background section, a citation to support the statement in lines 60-62 would be important.
Level of interest: An article whose findings are important to those with closely related research interests

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.