Reviewer’s report

Title: Serum Immunoglobulin G, M and A Response to Cryptosporidium parvum in Cryptosporidium-HIV Co-infected Patients

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Reviewer: Jeffrey W Priest

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Kaushik, K., et al. BioMed Central

Serum immunoglobulin G, M, and A response to Cryptosporidium parvum in Cryptosporidium-HIV co-infected patients.

Authors have used a crude soluble antigen ELISA to examine IgG, IgM, and IgA antibody responses in HIV infected and HIV negative individuals with and without Cryptosporidium infection. Sensitivities range from 19% for IgM to 100% for IgG. Specificities are reported to be >87.7%. No statistically significant correlations were noted between presence of an antibody response and either diarrhea or CD4 count (for HIV positive individuals).

Although the numbers of patients tend to be small, several groups have previously examined antibody responses to Cryptosporidium in HIV positive individuals. Gomez Morales et al. (JI, 1992) examined IgM and IgG in Italian HIV patients and found that 95% of stool confirmed individuals had one or both antibodies in their serum. Caputo et al. (Epidemiol. Infect., 1999) examined sera from HIV positive patients from Australia using the minigel Western blot format. References 24, 25, 26 and 32 also report data from HIV positive individuals. The authors claim that theirs is the first such report from India (page 5, line 2-3).

Major comments requiring revision:

1. How do the authors know that their patients were infected with C. parvum as stated in the title? Did they do PCR to rule out C. felis, C. hominis, etc.?

2. My major concern has to do with the sensitivity of the crude antigen ELISA and the determination of the cutoff value. Although this assay has been used in the past, a report by Frost et al. (Epidemiol. Infect., 1998) and a recent review by David Casemore (J. Water and Health, 2006) suggest that crude antigen ELISA data may need to be interpreted with care. Low levels of antibody may not be detected by the ELISA.

3. The authors use 5 control sera from supposed Cryptosporidium-negative, healthy individuals to establish a cutoff (mean + 2SD) (page 7, line11). My experience and literature reports (i.e. Frost et al., Ann. Epidemiol., 2004) suggest that 60-70% of the population has some level of antibody to specific C. parvum antigens. Hence the cutoff may be set too high, and the reported high specificities in Table 2 may be incorrect. What is the cutoff value?

4. The authors do not report response ODs for their ELISA assay. A 30 min
incubation at a serum dilution of 1:20 with 1 microgram of antigen in the ELISA well, in my experience, will yield a very high OD. If the OD values are so high, can there be any discrimination in response?

5. When were sera collected from cryptosporidiosis patients relative to onset of infection? The timing of collection can have a major impact on the levels of IgA and IgM (Moss et al., JID, 1998).

6. Authors fail to compare their results to those of Frost et al. (JID, 2005). Frost et al. reported that a strong IgG antibody response to the 27-kDa antigen was associated with a reduced risk of diarrhea in HIV positive patients in Australia.

**Level of interest:** An article of limited interest

**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.