Author's response to reviews

Title: Primary gamma-herpesviral infection in Zambian children.

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Version: 3 Date: 16 March 2010

Author's response to reviews: see over
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Version: 2 Date: 16 March 2010

Author's response to reviews: see over
Reviewer's report

**Title:** Primary gamma-herpesviral infection in Zambian children.
**Version:** 1  **Date:** 16 February 2010

**Reviewer:** Denise whitby

**Reviewer's report:**
Minhas et al describe a study of primary infection of a well characterized cohort of Zambian children with HHV-8 and EBV. The report is well written and clear and the methodology described sound. This is an important study as little has been reported to date on primary infection with HHV-8, especially in HIV and HHV-8 endemic regions such as Zambia where HHV-8 related disease is a major public health issue. The authors report that EBV infection occurs more frequently in children at 12 months of age in this cohort and the infection by the two gammaherpesviruses is independent. The association of primary infection by HHV-8 with rash substantiates a previous much smaller study. The importance of HIV-1 infection as a risk factor for HHV-8 primary infection as reported here cannot be overstated as this has profound public health implications for resource limited regions such as Zambia. The authors are to be congratulated for producing such a thoughtful and thorough study that substantially advances our understanding of this important topic.

- **Reviewer # 1 has suggested no changes for the manuscript**

**Level of interest:** An article of outstanding merit and interest in its field

**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
Reviewer's report
Title: Primary gamma-herpesviral infection in Zambian children.

Version: 1 Date: 24 February 2010

Reviewer: Chun Lu

Reviewer's report:
Re: “Primary gamma-herpesviral infection in Zambian children”

General Comments:
Minhas et al have assembled a set of data to compare the natural history of infection by EBV and HHV-8 along with the clinical manifestations and risk factors that are associated with early childhood infection in Zambia, which is an endemic area. It was suggested that there is no correlation between EBV and HHV-8 infections. Infection by one does not increase the susceptibility for the second virus. Primary HHV-8 and EBV infection in early childhood may clinically present as rash but remains largely asymptomatic and may remain undetected in this population. This study is potentially significant if the following questions can be addressed.

Major Points
1. With regard to HHV-8 test, the authors used BC-3 and baculovirus-infected Sf9 cells as known antigen with monoclonal-enhanced immunofluorescence assay (mIFAs). Did you compare this mIFAs with the other group-used methods, and what the result is?

   - Several laboratories have developed in-house assays which include ELISAs and IFAs which target different HHV-8 antigens. While we have not compared this assay with other assays, we have previously reported that our assay has a high sensitivity (94%) and specificity (96%) (Minhas et al, Clinical and Vaccine Immunology; 2008, 15: 1259-1264).

2. Why you didn’t use the Sf9 cells which were simultaneously infected by ORF65, K8.1A, and ORF73 baculovirus?

   - Sf-9 cells were infected individually by ORF65, K8.1A and ORF73 in order to maximize the level of protein production of each antigen. We have clarified this now by adding “to maximize the level of proteins produced”. (Underlined text on Page 6).

Minor Points
1. With the respect to relationship between HHV-8 and EBV, some literature indicated HHV-8 and EBV these two viruses can influence their replication for each other, while they simultaneously infected one cells, such as BC-1 and
JSC-1 cells. Therefore, some analyses and interpretation should be added to discussion.

The aim of this report was to investigate primary infection of HHV-8 and EBV in Zambian children. The reviewer does raise an interesting point which is relevant to events that may occur after primary infection and associated with disease progression. It has been reported that HHV-8 and EBV may interact at the molecular level and may promote the establishment of latency.

The following sentence has now been added to the discussion section “It is possible that dual infection may affect the disease progression because it has been reported that HHV-8 and EBV may interact at the molecular level and may promote the establishment of latency”. (Underlined text on Page 15).

In sum, the ms can be further considered.