Author's response to reviews

Title: Examining strain diversity and phylogeography in relation to an unusual epidemic pattern of respiratory syncytial virus (RSV) in a long-term refugee camp in Kenya

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Author's response to reviews: see over
We greatly appreciate the Reviewer’s comments. Our responses are given to each of the reviewer’s comments (italicized).

**REVIEWER 1**

**1.1** The unusual epidemiology is already described in a previous paper. The authors find no clues in a 620bp fragment (HVR2 of G) of the viral genomes/phylogenetics to account for this, but do not look at anything else, specifically no information on the sampled patients is presented... how many ILI, how many SARI, were all patients typical RSV patients, i.e. young children (<2) with bronchiolitis?

**Our response**

This question and similarly 1.2 and 1.3 below are certainly of interest. However, we feel that analysis of clinical and demographic features in relation to the epidemic behaviour goes beyond this paper as it is primarily an investigation of molecular phylogeny. We also suggest this would add considerably to the complexity of the paper since the analysis might require stratification by major-minor epidemic, by year, and by RSV group. One of the authors (JAA) is preparing a manuscript that explores the association between clinical, demographic and climatic factors and the temporal patterns of RSV in this population. For these various reasons we prefer not to develop this aspect in the present paper.

Nonetheless, to provide some description of case characteristics we have provided summary information (P10l8-16) on the sampled patients for the variables patient type (SARI or ILI), age, sex and number of days of illness prior to presentation at the facility. Further detail is provided in the earlier related publication (Ahmed et al., 2012).

**1.2** The authors philosophize whether the population build up and increasing numbers of susceptibles in the camp could be a factor.

**Our response**

See response to 1.1 above.
1.3 Were the patients’ characteristics of all the different peaks all similar?

Our response

See response to 1.1 above.

1.4 It would be informative if the authors could add additional information about known RSV seasonality in the region (in addition to the unpublished data from Kilifi)... are we seeing overlapping epidemiology of two different seasonal patterns?

Our response

This does in fact seem to be the case. We have included a section addressing this on P15l7-15.

1.5 P8 (ii) Definition of clades and sub-clades: more information on definitions of RSV genotypes is needed here, how have clades and sub-clades been defined by other authors, what does ICTV say? Authors need to argue why it is acceptable to adopt similar strategies for RSV (an obligatory human virus) as for avian influenza (a rapidly evolving virus with multiple avian – and mammalian dead-end - hosts)

Our response

Currently, there is no consensus on the criteria to define RSV genotypes, clades or sub-clades and ICTV does not provide guidance on this. The most commonly used system for classifying RSV strains was developed about a decade ago (Peret et al, 1998 and 2000) on the basis of phylogenetic clustering of historical strains and viruses from the early 1990s based on sequences of the hypervariable region of the RSV G-gene. Due to the rapid evolution of this specific region, distinguishing diversified strains within these genotypes 20 years hence is important.

The system developed by the Avian influenza researchers was adopted in the naming of the Dadaab RSV strains because of the way they assigned new names to current viruses of diversified genotypes which we considered appropriate: It assigns new names to the current viruses by taking into account their ancestral genotypes e.g. GA2 was the original genotype, and a recent form is called GA2.1 to reflect that it is has diversified from GA2. The rates of evolution and timelines of diversification may differ between the two viruses but their naming system
allows one to quickly identify where a new virus clade has come from. We have inserted a brief explanation of the rationale in the text P9l4-6

1.6 P7 l8-9 (reverse transcriptase [RT]) – between parentheses, as adenovirus is a DNA virus

Our response
We concur and have put “reverse transcriptase [RT]” in parentheses. P8l6-8

1.7 P7 l11-14 change to: only samples with a Ct value of 30 or lower were used.

Our response
We agree with the reviewer and have amended the sentence. P7l11-13.

1.8 P8 l1-9: specify primers used for sequencing reactions and whether sequencing was done bidirectional

Our response
We have clarified that the sequencing was bidirectional. The primer sequences have been previously described and rather than report these again in full, we have added the references to this in the manuscript P8l6-8.

1.9 P10 l7-8: delete “possibly due to low viral load as their CT values were close to 30”

Our response
We have deleted this statement as requested.

1.10 P10 l14-25: can the authors add information on RSV seasonality among hospitalized children in Kenya / Somalia

Our response
We have included a section in the discussion on RSV seasonality in Kenya on P15l7-15.
REVIEWER 2

2.1 The authors do not show any epidemiological characteristics for the recruited patients. The reviewer believe that basic characteristics of the patients who study samples obtained from as such sex distribution, average age are essential to be shown. In addition to the basic characteristics, the authors need to show how many samples were from ILI or SARI. If the authors could divide all cases into ILI and SARI as just numbers, the information is useful for readers who are interested in RSV epidemiology.

Our response
We have added summary information on the sampled patients in the results section (P10 |8-16). We were attempting to avoid overloading this paper with the clinical and demographic information as this had been given in much detail in a previous publication (Ahmed et al., 2012).

2.2 In the RSV group temporal patterns in results section from line 13 in page 10, definition of “major epidemic” is unclear from the Fig 2 and Table 1. Certainly the author described the definition major epidemic as “if during the epidemic period >15 cases were identified during any month”. However, when reader reads result section and see just Table 1, they should not recognize which epidemic is major or minor because no monthly numbers are shown in Table 1. Even in Fig 2, the readers might be confused to see which is major or not. If the authors add a line cases 15 on the Fig 2, it might be improved. This recognition should be important for readers because the authors discussed unusual biannual epidemics in the later part.

Our response
We have added a line across Figure 2 at 15 cases that would help the reader to quickly distinguish the minor and major epidemics. A hash symbol (#) has also been added to help identify the major epidemics within Table 1 and this is explained in the footnotes..

2.3 The authors discussed on the reason for unusual biannual epidemic pattern in the camp, and they suggest “the frequency of new strain introductions into the camp may not have been high compared to stable populations...” which seems to be
correct but the conclusion was opposite. This discussion is very important to think about critical factors to affect RSV circulation pattern and transmission. In the discussion, something lacks which is even for further future study. Climate factors should play major role for RSV circulation as the authors know. In tropical/subtropical zone, RSV outbreak occur during whole year especially in rainy season from the previous studies. How was climate in Dadaab during study period? The author showed the example in Kilifi which is located 500km far from Dadaab. However, Kilifi is just near by ocean, Dadaab is costal but not just near the ocean. The climate should be different among them. Thus, it is no wonder that the outbreak pattern was different among them when they consider from view of climate factors.

Our response

We have drawn the attention of the reader to the epidemic pattern difference between the Eastern and Western regions of Kenya, which may have a bearing on the observed pattern in Daadab. However, the precise underlying reason why Daadab lies epidemiologically in between the East and West is unknown. As the reviewer has pointed out, climatic factors may be of significance but this forms a basis for future study by JAA. P15l 7-15.

2.4 In the line 16 in the page 11, please correct the term as below:
in Nigaata, Japan >>> in Niigata, Japan

Our response

We have corrected this.

2.5 From the line 26 to 27 in the page 12, the authors mentioned about ON1 strain which is a GA2 variant containing a72 nucleotide duplication. The reviewer recommends to show it as “ON1 strain” because now its identified as “ON1 strain” in the world. The reader may easily recognize ON1 strain rather than “the strain including 72 nucleotide duplication”. And authors may know the ON1 strain were detected from South Africa recently. The authors may feel free to include the South Africa strains into their analyses because manuscript submission may be earlier than publication of the South Africa strains.

Our response
We have added a description of the 72-nucleotide duplication GA2 variant as the ON1 strain. However, the recently published ON1 strains from South Africa could not be included in this analysis as they were detected in 2012 yet this Daadab study was interested in contemporaneous sequences from 2007-2011.

2.6 In the similar reason to 1, the reviewer recommends to show the GA2(2.2) as NA1, or show both concurrently. The readers may easily understand “NA1” rather than just “GA2(2.2).

Our response
We want to draw the attention of the reviewer to note that the notations used herein, i.e. GA2(2.1) and GA2(2.2), help in showing the diversification observed within the GA2 strains from Daadab on the phylogenetic tree. We agree with the reviewer's comments and have therefore described GA2(2.2) strains to be closely related to the NA1 strain on P11113 and also shown both concurrently within the manuscript, where appropriate, for easier interpretation.

REFERENCES

